APPROVAL SHEET

Title of Thesis: "Environmental Enrichment, Performance, and Brain Injury in Male and Female Rats"

Name of Candidate: Brenda M. Elliott
Doctor of Philosophy Degree
2004

Thesis and Abstract Approved:

_____ _____
Joseph McCabe, Ph.D. Date
Committee Chairman

_____ _____
Neil E. Grunberg, Ph.D. Date
Committee Member

_____ _____
Wendy A. Law, Ph.D. Date
Committee Member

_____ _____
Martha M. Faraday, Ph.D. Date
Committee Member

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
2004		**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Environmental Enrichment, Performance, and Brain Injury in Male and Female Rats	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Uniformed Services University of the Health Sciences (USUHS),4301 Jones Bridge Road,Bethesda,MD,20814-4799**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
see report

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**		**227**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

> "Environmental Enrichment, Performance, and Brain Injury in Male and Female Rats"

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violations.

Brenda M. Elliott
Department of Medical and Clinical Psychology
Uniformed Services University of the Health Sciences

ABSTRACT

Title of Thesis: "Environmental Enrichment, Performance, and Brain Injury in Male and Female Rats"

Author: Brenda M. Elliott, Doctor of Philosophy, 2004

Thesis directed by: Neil E. Grunberg, Ph.D.

Professor

Department of Medical and Clinical Psychology

Environmental enrichment affects performance of intact organisms and improves recovery from brain injury. The extent to which physical vs. social aspects of enriched environments separately contribute to superior performance or the extent to which males and females differ in their response to enrichment has not been examined previously.

The goals of this doctoral research were to examine the separate and combined effects of social enrichment (SE) and physical enrichment (PE) on cognitive performance of neurologically-intact and brain-injured rats and to determine if there were gender differences in these effects. Measures of basic (*i.e.*, locomotor habituation and ASR/PPI) and complex cognitive processing (*i.e.*, passive avoidance, Morris water maze) were used to determine if enrichment affected performance on simple and complex cognitive measures. Experiment I examined the effects of enrichment on performance of 192 intact animals. Experiment II examined the effect of enrichment on performance of 96 injured animals.

The major findings from the current study were: 1) social enrichment has the greatest effect to improve performance for both intact and injured animals; 2) the effects of enrichment overall generally appear to be greater for males than for females; 3) overall enrichment has the greatest beneficial effect on tasks that require the active processing of information. These findings replicate and extend previous work on enrichment and may have important implications for educational programming and brain injury rehabilitation.

Environmental Enrichment, Performance, and Brain Injury in Male and Female Rats

by

Brenda M. Elliott

Doctoral Dissertation submitted to the Faculty of the

Department of Medical and Clinical Psychology

Graduate Program of the Uniformed Services University

of the Health Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy, 2004

DEDICATION

This work is dedicated to

the memory of William P. Elliott

whose kindness, generosity, and sense of humor

enriched the lives of all who knew him

ACKNOWLEDGEMENTS

Don't aim for success if you want it; just do what you love and believe in it; and it will come naturally. –David Frost

The past several years have been an incredible journey. When I began this journey, my goal was to become a more effective clinician and scientist. Along the way, I have gained so much more. My successful completion of this journey would not have been possible without the knowledge, support, and guidance of many people. In particular, I would like to thank the Medical and Clinical Psychology faculty, especially Dr. Michael Feuerstein, whose support has made my dream of becoming a true scientist- practitioner a reality.

I also want to thank my colleagues and friends at USUSHS, especially the members of the Grunberg lab (Bonnie Yatko, Jennifer Phillips, Joshua Tomchesson, Hirsch Davis, Gina Minich, Sarah Shafer, and Ashley Myracle) for their patience and support throughout this process. I am especially grateful to Jennifer Phillips, whose generosity and skill made this work possible. I thank the members of my committee (Drs. Joseph McCabe, Neil Grunberg, Wendy Law, and Martha Faraday) whose time, commitment, and insightful comments on this project were invaluable. Dr. Martha Faraday, in particular, was an important role model for me, especially during the early part of this process. Dr. Wendy Law has been a great support to me from day one.

Several other individuals made significant contributions to this work. Dr. Geoffrey Ling and the members of his lab, especially Elle Lee, graciously allowed me use their fluid percussion device and trained me in the proper brain-injury procedures. The Laboratory of Animal Medicine staff, especially Carrie Hibler, Jonathon Smith, and Gina Fulton, always made sure I had enough cages and toys for my project and took amazing care of my animals during this entire project. The Uniformed Services University, Henry M. Jackson Foundation, and the National Institute of Neurological Disorder and Stroke provided me with continuous funding through this entire process.

I would like to thank all of my family and friends who encouraged me and supported me, giving me the courage to press on and continually ensuring that I found the time to relax and enjoy life.

Finally, I would like to extend my deepest gratitude to the two individuals who truly made this journey possible. Neil Grunberg, my mentor, has been my guide every step of the way. His endless gifts have made me a more thoughtful scientist and a more empathic clinician. By always encouraging me to strive for more, he has taught me to believe in myself and has allowed me to accomplish more than I ever thought possible. Phil Elliott, my husband, has been my faithful companion on this journey, trusting me, supporting me, and loving me each and every day. His love of life, generous spirit, and kindness are infectious and unparalleled. My life is more complete because of him.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Overview

Cognition can be defined as the intellectual processes through which information is obtained, transformed, stored, retrieved, and used (Simpson, 1989). Examples of specific cognitive processes include attention, memory, visual-spatial organization and analysis, problem solving, planning, and organization. All of these processes affect performance and behavior and are influenced by environmental and biological factors. These factors together and separately affect the organization, development, and manifestation of cognition in intact and injured brains.

Environment and Cognitive Performance

Clinical and laboratory evidence suggests that environmental stimulation and experience are necessary for healthy brain development and may affect cognitive recovery following brain injury. Children raised in impoverished environments exhibit impairments in cognitive and behavioral functioning, whereas children raised in highly stimulating or enriched environments exhibit enhanced behavioral and cognitive outcomes (Kaler & Freeman, 1994; Joseph, 1999). Animal studies have reported similar findings, thereby providing valid models to evaluate these factors under controlled experimental conditions. Specifically, enriched environments, characterized by the presence of physical objects and the opportunity for social interaction, have shown profound and long-lasting positive behavioral and physiological consequences and even appear to “protect” the organism from age-related deficits in learning (Diamond, 1967; Sanchez, Ladd, & Plotsky, 2001). In

contrast, those animals that were reared in impoverished environments, devoid of physical and social stimulation, demonstrate disruptions in cognition and behavior. The beneficial effects of enriched environments also have been reported in brain-injured humans and animals with better recoveries occurring in enriched environments (Passineau, Green, & Dietrich, 2001; Taylor *et al*., 2002). Together, these findings suggest that a stimulating or enriched environment is essential for healthy brain development and may potentially enhance recovery following brain injury. However, the relative contributions of social vs. physical environmental enrichment to improve performance is unknown.

Biology and Cognitive Performance

Gender is a major biologically-based variable that affects cognition; Men and women differ in cognitive abilities. Specifically, considerable evidence suggests that, on average, men are superior to women on visual-spatial tasks, and women are superior to men on measures of verbal fluency, verbal memory, perceptual motor speed, and some fine motor skills (Coltheart, Hull, & Slater, 1975; Maccoby & Jacklin, 1978; Halpern, 1992; Kimura, 1992; Springer & Deutsch, 1998).

Sex differences in cognitive abilities and cognitive recovery also have been reported in neurologically-compromised populations. For example, female infants show better cognitive recovery than male infants following intracranial hemorrhage and respiratory distress (Raz, Goldstein, Hopkins, & Lauterbach, 1994; Lauterbach, Raz, & Sander, 2001). Following head injury, female children perform better on cognitive tasks than do male children (Donders & Woodward, 2003). Among adults,

women exhibit better verbal recovery after left hemisphere stroke than do males and reportedly exhibit overall better recovery than males following brain injury (Grosswasser, Cohen, & Keren, 1998).

These performance differences between the sexes may be explained by differences in brain architecture. Gron and colleagues (2000), for example, used functional MRI to observe brain activation in men and women as they made their way through a three-dimensional virtual-reality maze. Men and women used different neuroanatomical regions to complete the task. For men there was activation of the left hippocampus during the task, whereas for females the right parietal and right prefrontal cortex were activated. Human gender differences in brain activation during a verbal working memory task also have been reported (Speck, Ernst, & Braun *et al.,* 2000). Specifically, males exhibit primary right hemisphere activation of the lateral prefrontal cortex and parietal cortex, whereas females demonstrate greater left-hemisphere activation of these same brain regions. Together, these findings suggest that males and females rely on different brain regions to complete cognitive tasks. Whether these differences in functional brain organization alter how males and females respond to the environment is less clear, but may be relevant to understand further gender differences in cognitive performance or gender differences in brain-injury recovery.

Another possible explanation for reported sex differences in cognitive abilities is sex hormones. Sex hormones are secreted by the testes in males and the ovaries in females, and by the adrenal glands in both sexes. Although males and females produce the same hormones (*i.e.,* estrogen, progesterone, and testosterone), the

relative concentrations of these hormones differ in the sexes and across the life cycle. These differences in relative concentrations between genders are thought to underlie many of the reported differences in cognitive ability. Testosterone levels, which are higher in males, correlate with spatial ability—tasks on which males traditionally excel (Kimura, 1992). In women, cognitive performance fluctuates throughout the menstrual cycle. Specifically, at mid-cycle, when estrogen and progesterone levels are high, females perform better on verbal fluency and manual dexterity tasks. In contrast, performance on spatial tasks is better during the low-estrogen part of the cycle (Kimura, 1992). Together, these findings suggest that hormones contribute, in part, to cognitive differences between the sexes.

Environment and Gender Interactions on Cognitive Performance

Few studies have examined the interaction of gender and specific environmental factors on cognitive performance in either intact or injured brains. Based on the findings of marked benefits from exposure to physically and socially-enriched environments described previously, contributions from any gender interactions with these effects would be important to understand. Specifically, differences in how males and females respond to social and physical aspects of the environment may have important implications for understanding how to optimize or to improve performance in neurologically-intact individuals. For example, determining whether males and females differ in their sensitivity to social and physical aspects of the environment may be critical for the development of educational programs. Further, if gender interacts with the environment to affect

cognitive performance, then such an interaction also might affect cognitive recovery following brain-injury or neurological illness. Understanding the gender-environmental interaction, therefore, may have important clinical implications for the recovery and treatment of neurologically-impaired individuals.

The specific aims of this doctoral research were to: 1) determine to what extent the components of the enriched environment (*i.e.,* social vs. physical) differentially enhance cognitive performance in intact brains; 2) determine whether the effects of enrichment on performance differ in males and females; 3) determine which components of the enriched environment best facilitate recovery of cognitive functioning following brain injury; and 4) determine whether the effects of the environment to enhance recovery of function following brain injury differ in males and females. The specific aims were investigated by comparing the separate and combined effects of social and physical enrichment on measures of habituation, attentional processing, simple memory, and complex spatial memory in intact and brain-injured male and female Sprague-Dawley rats. Male and female rats were included to determine whether the effects of enrichment on the selected cognitive measures differ depending on animal gender. A variety of behavioral measures were included to provide a comprehensive picture of how enrichment influences recovery across various levels of cognitive complexity

The remainder of the introduction reviews relevant background material including: 1) environmental enrichment effects on cognitive performance in intact animals, 2) gender differences in enrichment effects on cognitive performance in intact animals; 3) traumatic brain injury and cognitive performance in human and

animal models of traumatic brain injury; 4) environmental effects on cognitive performance in brain-injured animals, 5) gender differences in brain injury recovery in human and animal models, 6) and the rationale for the dependent variables used in this study.

Environment and Cognitive Performance: Early Support for Enrichment Effects

Charles Darwin (1874) reported that the brains of domestic rabbits were considerably smaller compared to the brains of wild rabbits. Darwin argued that domestically-reared animals did not exert their intellect, instincts, and senses as much as animals did in the wild, and that their reduced brain size was a consequence of a deprived environment. It was not until almost a century later that this concept reemerged and began to be tested experimentally. Donald Hebb (1947) is credited with providing some of the earliest evidence that the environment affects performance and cognitive development.

Hebb (1947) reported that rats which he took home from the laboratory and treated as pets later performed better on a maze learning task than did their littermates which had remained in the laboratory. Based on these observations, Hebb postulated that brains must change in response to new information. More specifically, Hebb postulated that if multiple nerve cells receive a stimulus at the same time that causes them to fire, then eventually the number of connections at that site will increase, resulting in enhanced behavioral and functional outcomes and more rapid learning of new information.

Following Hebb's serendipitous observation, a group of investigators at Berkeley introduced environmental enrichment as a testable scientific concept (Rosenzweig, Krech, Bennett, & Diamond, 1962). This group, led largely by Diamond and Rosenzweig, compared the brains of rats raised in an enriched environment to rats raised in isolation and found that enriched rats developed greater cerebral cortex weights, greater capillary diameter in the cortex, and greater total activity of brain acetylcholinesterase when compared to rats raised in isolation (Rosenzweig *et al.,* 1962; Rosenzweig, 1966). Diamond's and Rosenzweig's discoveries soon were followed by studies suggesting that stimulating environmental conditions or "enriched environments" increase the size and weight of the cortex, increase neuron size, and enhance dendritic branching, gliogenesis, synapse formation, acetylcholine synthesis, and protein levels (Altman & Das, 1964; Diamond, 1967; Rosenzweig, Bennett, & Diamond, 1972**;** Greenough, 1975; Greenough, Black, & Wallace, 1987). These early studies provide the first experimental evidence that environmental enrichment affects brain development and function.

Enriched environments refer to the amount of physical or social stimulation that is available in the environment. Enriched environments differ from impoverished or standard environments in both the number of animals per cage and the number of objects per cage. For rats, the classic enriched environment involves housing rats in groups (3-12) to provide opportunities for social interaction (social enrichment) and including toys and objects to provide opportunities for physical stimulation (physical enrichment) (Rosenzweig & Bennett, 1996; Woodcock &

Richardson, 2000). Most studies of enriched environments provide both physical enrichment (PE) and social enrichment (SE) and, therefore, are labeled as "PESE" in this doctoral dissertation report. In contrast, the standard or non-enriched environment consists of housing animals individually without physical objects (Kolb, Forgie, Gibb, Gorny, & Rowntree, 1998; Van Praag, Kempermann, & Gage, 1999; Varty, Paulus, Braff, & Geyer, 2000). The standard environment, that does not provide physical enrichment or social enrichment, is referred to as "NPESE" in this report.

Extensive research suggests that environmental enrichment enhances cognitive performance in neurologically-intact animals and may improve recovery of cognitive functioning in neurologically-impaired organisms. Animals reared in enriched environments (PESE) exhibit better cognitive performance (Van Praag *et al.,* 1999; Varty *et al.,* 2000; Smith, 1972, Gardner, Boitano, Mancino, & D'Amico, 1975; Daniel, Roberts, & Dohanich, 1999) than animals reared in standard non-enriched environments (NPESE). For example, rats raised in a PESE environment are better able to discriminate between a conditioning context and a similar but distinct context than are NPESE-reared rats (Woodcock & Richardson, 2000). Additionally, PESE rats adapt more rapidly to a novel environment than do NPESE-reared animals (Varty *et al.,* 2000). PESE animals also perform better than SE and NPESE-reared rats on learning and memory tasks, including the Morris water maze (Daniel *et al.,* 1999; Pham, Ickes, Albeck, Soderstrom, & Mohammed, 1999; Williams *et al.,* 2001). In addition, NPESE-reared rats perform worse than PESE and SE rats on memory and learning tasks.

The existing literature on rat models of environmental enrichment is extensive. Unfortunately, there are two major limitations of the published work. First, the extent to which physical vs. social aspects of enrichment separately contribute to superior performance has not been examined thoroughly. Second, rodent models of environmental enrichment have used primarily male subjects. Male and female rats differ in learning and memory performance (Brandeis, Brandys, & Yehuda, 1989; Hooge & DeDeyn, 2001). Further, human females reportedly recover faster from neurological injury than do males (Roof & Hall, 2000; de Courten-Meyers, 1999). Whether environmental conditions (social vs. physical) interact with gender to enhance learning and cognitive performance or affect recovery from neurological injury has not been examined thoroughly. Identifying whether specific aspects of the environment (social vs. physical) interact with gender may help to explain gender differences in cognitive ability and recovery and may aid in the development of educational programs for neurologically-intact individuals or rehabilitation programs for neurologically-impaired individuals.

Enrichment Effects on Cognitive Performance: Gender Differences

Studies examining the effects of enriched environments on cognitive performance have used primarily male rats as subjects. A few studies have compared the performance of male and female rats raised in a PESE environment to males and females reared in an NPESE environment. Male and female rats raised in PESE environments perform better on a spatial memory task than do rats raised in NPESE (e.g., Einon, 1980). In addition, male and female rats raised in PESE

environments make fewer errors on memory tasks than do rats raised in NPESE environments (Seymore, Dou, & Juraska, 1996). Males did not differ from females in their response to enrichment in these studies. A few studies that examined the performance of females alone on tasks of spatial memory have obtained similar results with regard to enrichment effects (e.g., Daniel *et al*., 1999).

Work conducted in our laboratory has examined the effects of group housing (*i.e*., animals were housed in groups of six) on locomotion, feeding, acoustic startle, and pre-pulse inhibition (PPI) in male and female Sprague-Dawley rats (Brown & Grunberg, 1995; Faraday, Rahman, Scheufele, & Grunberg, 1998; Faraday, Scheufele, Rahman, & Grunberg, 1999). These studies suggest that females are more sensitive than are males to the behavior-altering effects of group housing (*i.e.,* social enrichment).

Experiment I of this doctoral dissertation research was conducted to examine the effects of environment on cognitive performance in intact animals. Specifically, this experiment gathered data regarding the effects of NPESE, SE, PE, and PESE environments on various aspects of behavior and cognitive performance (*i.e.,* open-field activity, acoustic startle response, pre-pulse inhibition, and Morris water maze) in neurologically-intact male and female Sprague-Dawley rats (see Experiment I: Methods). The specific goals of Experiment I were to: 1) determine the feasibility of the procedures for producing the physical (PE), social (SE), and combined (PESE) enrichment conditions, 2) establish the logistical feasibility of housing and handling subjects for Experiment II, 3) collect data that could provide a basis for comparison in brain-injured animals, 4) determine whether enriched environments affect

cognitive processes in ways that might aid in the development of educational programs designed to optimize cognitive performance

If environmental enrichment can enhance the cognitive performance of intact animals, then it may be possible to use enriched environments to improve cognitive performance that has been altered secondary to illness or injury, such as cognitive dysfunction resulting from traumatic brain injury. Traumatic brain injury (TBI) results in significant impairments in cognitive functioning. Further, males and females reportedly differ in TBI recovery. Experiment II was conducted to examine the effects of physical and social enrichment on cognitive performance following brain injury and to determine whether there are gender differences in these effects.

The literature related to the nature, impact, and intervention methods of TBI next will be reviewed. This discussion will be followed by the clinical and experimental findings related to environmental enrichment in TBI. An overview of the current research will be provided, including the basis and rationale for the proposed research in relation to unanswered questions in the existing literature.

Traumatic Brain Injury: Description and Epidemiology

Traumatic brain injury (TBI) is the leading cause of death and disability among children and young adults in the United States (CDC, 2002). Each year 1-2 million Americans are brain-injured. Of those individuals who survive, approximately 80,000 suffer long-term impairments in physical, cognitive, and psychosocial functioning, despite rehabilitation efforts (CDC, 2002). Currently, over 5 million men, women, and children are estimated to be living with a permanent TBI-related

disability in the United States (CDC, 2002). Although primary prevention of traumatic brain injury is critical, an effective approach to traumatic brain injury also requires the development of programs and interventions to minimize adverse outcomes and maximize recovery of function among brain-injured individuals.

Broadly, a traumatic brain injury can be defined as injury to the brain resulting from externally-inflicted trauma. Traumatic brain injuries principally result from vehicular incidents, falls, and sports injuries (NIH, 1999). Injuries which occur in this manner typically cause extensive damage to the brain without penetration of the skull and are commonly referred to as closed-head injuries. By definition, a closed head injury occurs when the head strikes an object or is struck by an object at high speed (*e.g.,* dashboard, floor, or flying object).

When the head is struck at a high velocity, the impact propels the brain inside the skull, causing damage to the brain both at the point of impact (the *coup*) and at an area of the brain diametrically opposite to the point of impact (the *contre coup*). Movement of the brain in this manner also creates extensive and diffuse damage within the cortical and subcortical white matter as a result of rotational forces that cause axonal shearing (Katz & Alexander, 1994; Mittl *et al*., 1994). There is frequently damage to the hippocampus with closed-head injuries because of its vulnerability to ischemia and because of its position in the temporal lobe at the end of long fiber tracks (Kotapka, Grahm, Adams, & Gennarelli, 1994).

Given the extent of damage to the brain resulting from a closed head injury, it is not surprising that the resulting functional deficits are complex and widespread, resulting in changes in neurological, social, emotional, behavioral, and cognitive

functioning. Of all the residual deficits following traumatic brain injury, disruptions of cognition and behavior are the primary contributors to disability in two-thirds of patients evaluated at six months post-injury (Jennett, Snoek, Bond, & Brooks, 1981). Further, the degree of impairments in cognitive or behavioral functions are closely related to the severity of injury (Brown & Levin, 2001).

TBI and Cognitive Performance

The cognitive consequences of brain injury are broad and most often parallel the extent of the damage to various brain regions. Some of the most common cognitive problems include: alterations in memory, attention, visual-spatial skills, and language. More complex loss of function may include disruptions in the ability to plan and organize behavior or respond appropriately to the environment. Changes in these aspects of cognition frequently interfere with an individual's ability to return to a premorbid level of functioning or to live independently. Maximizing recovery of function, therefore, may be the key to reducing post-injury disability.

Traumatic brain injury has a profound impact on learning and memory in humans (Hall & Bornstein, 1991). Deficits in memory are the most frequently-reported cognitive complaint following traumatic brain injury (Capruso & Levin, 1992). Specific memory impairments may include disruptions in the acquisition of new information (visual or verbal) or disruptions in the retrieval of previously learned information (Brown & Levin, 2001). The hippocampus is the neuroanatomical region responsible for most memory functions. Its subcortical location also puts it at a high

risk for ischemic injury as a result of rotational forces and may help to explain why memory disturbances are so common after brain injury.

Deficits in attention are the second most frequently-reported complaint of traumatic brain injury patients and are thought to underlie many of the other cognitive deficits experienced by brain injury survivors (van Zomeren & Brouwer, 1994; Nieman, Ruff, & Kramer, 1996). Deficits in attention commonly result from damage to the anterior frontal cortex and brainstem. The most common disorders of attention include: trouble sustaining attention, impaired selective attention and scanning, and poor shifting of attention between tasks. Generally, the degree of attentional deficit is related to the severity of the injury. The time course for recovery of attentional deficits may extend up to two years post-injury and in more severe injury may never fully improve to pre-injury levels (van Zomeren & Brouwer, 1994).

Executive functioning refers to the capacity of an individual to plan and organize behavior and to respond appropriately to the environment. Individuals with disruptions in executive functioning have difficulty completing goal-directed tasks or navigating effectively within the environment. Disruptions in executive functions are common after damage to the frontal lobes (Brown & Levin, 2001; Spikman, Deelman, & Van Zomeren, 2000). Similar to disruptions in attention and memory, disturbances in executive functions may significantly interfere with an individual's daily occupational and interpersonal functioning. Interventions designed to treat brain injury are often aimed at reducing the extent of disturbances in all of the cognitive domains affected by brain injury.

Traumatic Brain-Injury Treatments

Despite the wide-ranging and substantial effects of brain injury on an individual's functioning, the brain does have some capacity to recover and the extent of impairment can be reduced if proper treatment is administered. Treatments for brain-injury vary depending on the type of injury, but typically include both acute interventions aimed at stabilizing the patient and improving short-term outcomes and long-term interventions aimed at regaining lost function and improving long-term outcomes. Acute interventions may include surgery to control bleeding in and around the brain. Medications are used to prevent seizures and to reduce swelling and damage (NIH, 1999). Pharmacological agents also may be useful in a variety of affective and behavioral disturbances associated with TBI. However, their effects to ameliorate cognitive deficits are less clear.

After acute interventions have been employed and the patient is stabilized, rehabilitation strategies are introduced (NIH, 1999). Rehabilitation is an important part of the recovery process for a TBI patient. Rehabilitation is characterized by a multidisciplinary approach to treatment (*i.e.,* physical therapy, occupational therapy, speech therapy) that is designed to help patients understand and manage their disabilities in as normal an environment as possible (Diller, 1987). Ideally, rehabilitation programs should be individually designed based upon the patient's strengths and capacities and modified over time to meet the patient's changing needs (NIH, 1999). Rehabilitation procedures, although commonly employed, have had mixed results.

A potentially promising intervention for brain injury involves environmental enrichment. Research with animals suggests that post-injury environmental enrichment (PESE) can influence functional outcomes, tissue integrity, and overall recovery from brain injury (Passineau, Green, & Dietrich, 2001, Hamm, Lyeth, Jenkins, O'Dell, & Pike, 1993; Johansson & Ohlsson, 1996; Taylor *et al.,* 2002). Animals exposed to complex, highly stimulating, and social environments exhibit better functional outcomes, as measured by superior performance on cognitive-based tasks, than do animals recovering in standard non-enriched environments. Recent clinical reports support these findings.

TBI and Environmental Enrichment: Clinical Findings

The benefits of enriched environments on brain development in humans have been widely documented. Whether these findings extend to brain-injured individuals has been investigated recently. Following traumatic brain injury, children recovering in unfavorable family circumstances have less rapid short-term progress and more behavioral problems (*i.e.,* externalizing and internalizing behaviors) as measured by the Child Behavioral Checklist (CBCL) than do children recovering in socially-and physically-enriched environments (Taylor *et al.*, 2002).

Further, analyses of post-injury recovery in humans indicate that the pre-injury family environment consistently predicts the level of cognitive and behavioral functioning 12 months post-injury, with high-functioning families buffering the impact of injury and low-functioning families exacerbating the deficits (Yeates, *et al*., 1997). Together, these studies provide support for the potential therapeutic effects of

environmental enrichment on cognitive function following traumatic brain injury and may have important implications for brain injury rehabilitation.

Several questions, however, remain unanswered. First, what are the specific components of the environment (*i.e.,* physical or social) that are responsible for the observed effects? Second, do males and females differ in their responses to enrichment effects on brain injury recovery? The answers to these questions are important clinically to aid in the development of rehabilitative treatment programs and to tailor rehabilitative programs as needed to meet individual (*e.g.,* gender-specific) needs. Because ethical and logistical concerns limit the feasibility of studying these questions in humans, animal models of traumatic brain injury offer valuable alternatives to examine the relative contributions of these variables under strict, scientifically-controlled experimental procedures.

Animal studies complement human studies and allow detailed experimental examination of environmental influences on brain injury recovery. Unlike human studies, animal models allow for the direct manipulation of brain injury type and severity as well as the control of environmental conditions and additional factors that may influence recovery (*e.g.,* age and gender). Animal models, therefore, provide a unique opportunity to isolate and examine whether there are gender differences in the effects of environment on recovery and outcomes following traumatic brain injury. In addition, much of what is currently known about enriched environments comes from animal studies.

TBI and Environmental Enrichment: Experimental Evidence

Several studies have examined the potential for PESE and SE to facilitate recovery from brain injury in rats (Passineau *et al*., 2001, Kolb & Gibb, 1991; Johansson & Ohlsson, 1996). Most of these studies have compared the performance of PESE-housed animals post-injury to animals housed in a SE environment or in an N-PESE environment post-injury. The PESE environment has demonstrated the greatest impact on functional outcomes after experimental brain injuries (Passineau *et al*., 2001; Johansson & Ohlsson, 1996). For example, rats kept in a PESE environment following cerebral artery ligation performed better on motor tasks than did rats kept in isolation (Johansson & Ohlsson, 1996). Although rats recovering in the SE condition performed better than did rats recovering in isolation, rats recovering in the PESE environment exhibited the best performance overall (Johansson & Ohlsson, 1996). Similarly, following a fluid percussion injury, PESE-housed rats exhibited greater improvements in maze performance and motor coordination and integration than did N-PESE-housed rats (Passineau *et al*., 2001). Together, these studies suggest that a combination of physical and social enrichment improves recovery, but that social enrichment alone cannot account for the observed effects. Whether PE alone contributes to these effects has not been examined.

Based on the existing literature, it is not clear whether physical and social enrichment interact to produce the beneficial effects of the PESE environment. By manipulating both social and physical enrichment, the current research was

designed to clarify the specific types of enrichment that affect functional recovery. In addition, because previous studies have used primarily male subjects, males and females were included in this research to determine whether males and females respond differently to specific aspects of the environment. These potential gender effects were examined in both intact and brain-injured animals.

OVERVIEW OF PROPOSED WORK

The existing literature on rat models of environmental enrichment is extensive and suggests that environmental enrichment improves performance regardless of animal sex. The generalizability of the findings, however, is limited in two ways. First, in the majority of these studies rats reared in PESE environments were compared to SE or N-PESE rats. Consequently, the extent to which the specific aspects of the environment—social and physical—separately contribute to superior performance cannot be determined. Second, studies examining the effects of enrichment on brain injury recovery primarily have used tasks known to index complex cognitive processes (*i.e.,* spatial learning, spatial memory, and working memory). Brain injury, however, is known to affect both basic (*e.g.,* information processing, attention) and complex (*e.g.,* memory) cognitive functions. Whether differences exist in the effects of environmental conditions on more basic cognitive processes (*i.e.,* attention, auditory processing, behavioral adaptation) has not been examined but may be relevant to post-TBI human recovery. **Deficits observed in complex task performance may be the result of deficits in simpler processes. Therefore, it is important to assess learning at different levels of complexity.** Third, studies examining the effects of enrichment on brain injury recovery have

primarily used males as subjects. Males and females, however, are known to differ in recovery from brain injury. The extent to which environmental factors may contribute to these recovery differences has not been examined, but may have important implications for tailoring rehabilitation programs to maximize recovery for individual patients.

This research project, which consisted of two separate experiments, was designed to address these three limitations and to provide data to complement the existing literature. Experiment I compared the effects of N-PESE, PE, SE, and PESE on cognitive performance (open-field activity, acoustic startle reflex and prepulse inhibition, and Morris water maze) in neurologically-intact male and female Sprague-Dawley rats. Experiment II compared the same cognitive performance tasks plus passive avoidance in brain-injured Sprague-Dawley rats living in social, physical, or combined enrichment conditions. Gender differences were evaluated by comparing the response of male and female brain-injured rats. Basic and complex cognitive processes were evaluated by including a wide variety of behavioral measures. Specifically, the behavioral responses measured included basic unconditioned behaviors (*i.e.,* locomotion and the acoustic startle reflex and prepulse inhibition), a simple working memory task (*i.e.,* passive avoidance), and a complex spatial learning and memory performance task (*i.e.,* Morris water maze). These measures were included to provide a comprehensive picture of how enrichment influences recovery across various levels of cognitive complexity.

The goals of this project were to: 1) determine which components of the enriched environment have the greatest influence to enhance cognitive performance

in neurologically-intact animals, 2) determine whether males and females respond differently to specific components of the enriched environment to enhance cognitive performance, 3) determine which components of the enriched environment have the greatest influence to enhance recovery of cognitive functioning following brain injury; 4) determine whether males and females differ in their recovery from brain injury across various measures; and 5) determine whether brain-injured males and females respond differently to the effects of the environment on cognitive performance.

Rationale for Dependent Variables: Measures to index cognitive performance

Enrichment studies have utilized a variety of measures to index cognitive abilities. The most widely-used measures include: open field locomotor activity, acoustic startle response, pre-pulse inhibition of the acoustic startle, and the Morris water maze. These measures index different aspects of learning and memory. Locomotor activity and acoustic startle provide the simplest measures of learning—exploration and habituation to a novel environment/stimulus, respectively. Pre-pulse inhibition measures a higher form of information processing—attentional regulation or sensorimotor gating. Passive avoidance and the Morris water maze are more complex measures of learning and information processing – working and spatial memory, respectively. Together, these measures provide a comprehensive picture of how enrichment might influence learning at varying levels of complexity.

Enrichment effects on brain-injured animals have focused primarily on recovery of more complex cognitive functions (*e.g.,* Morris water maze, radial arm

maze). Only a few studies have examined enrichment effects on the recovery of more simple cognitive functions (*e.g.,* attention, information processing). Brain injury, however, is known to affect both simple and complex cognitive processes. Multiple behavioral measures, therefore, were included in this research to provide a more comprehensive picture of how enrichment affects brain injury recovery at various levels of cognitive performance.

Open-Field (locomotor) Activity

Open-field locomotion responses refer to an animal's behavior when placed in a non-home cage arena. Behaviors include activity in the horizontal plane, distance traveled, and rearing. Level of activity and frequency of rearing behaviors have been used to index the extent to which an animal habituates to a novel environment (Varty *et al.,* 2000; Bowling, Rowlett, & Bardo, 1993; Van Waas & Soffie, 1996). Habituation is the simplest form of learning and refers to the progressive reduction in response to an initially novel stimulus when the stimulus is repeatedly presented to a subject (Varty *et al.,* 2000). A decrease in overall activity or rearing behaviors is indicative of habituation or efficient processing of novel information. Absence of behavioral change over time reflects deficient environmental processing. Deficiencies in processing novel information may decrease learning rates and interfere with an organism's ability to adapt effectively to its environment. PESE-raised animals exhibit reduced locomotor activity, reduced exploration over time, and more circumscribed movements when compared to NPESE-raised animals (Varty *et al.,* 2000; Bowling *et al.,* 1993; Van Wass & Soffie,

1996; Paulus, Bakshi, & Geyer, 1998; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001). These patterns suggest that PESE enhances the ability of the animal to adjust its behavior in relation to the environment. In contrast, animals raised in NPESE exhibit hyperactivity and decreased habituation when compared to PESE or SE rats. Together, these findings suggest that PESE rats assimilate information from their environment and adapt more effectively to novel environments than do rats raised in NPESE.

Acoustic startle and Pre-Pulse Inhibition

The acoustic startle reflex (ASR) is a defensive reflex consisting of involuntary, muscular responses elicited by a sudden acoustic stimulus (Davis, 1984). Changes in startle responses are thought to reflect changes in reactivity or responsiveness to novel stimuli (Davis, 1984). In addition, ASR has been used to evaluate response habituation. Pre-pulse inhibition (PPI) of the ASR occurs when the startling stimulus is preceded by a non-startling stimulus by a short interval, resulting in reduced startle amplitude (Hoffman & Ison, 1980). PPI indexes an innate sensorimotor gating mechanism that operates at a non-volitional level. A few studies have examined the effects of environmental conditions on ASR and PPI (Geyer, Wilkinsion, Humby, & Robbins, 1993; Wilkinson *et al.,* 1994). PESE-reared rats exhibited increased ASR and normal PPI compared with SE-reared rats. Isolation-reared rats also exhibited increased ASR, but with reduced PPI relative to SE-reared rats. Studies examining the effects of PE alone on ASR, PPI, or startle habituation have not been reported.

Male rats exhibit greater startle reactivity, steeper habituation curves across trials, and increased PPI when compared to female rats (Lehmann, Pryce, & Feldon, 1999; Faraday & Grunberg, 2000). These results suggest that males and females differ in attentional processing and in the rates at which they process novel information. Specifically, males appear to adapt more quickly to novel stimuli. The extent to which males and females differ in their responses to specific aspects of the enriched environment (*i.e.,* social and physical) to affect ASR, PPI, and startle habituation has not been examined. Experiment I examined the effects of environmental enrichment to alter ASR, PPI, and startle habituation in intact male and female rats.

Examination of ASR and PPI in this project was important for several reasons. First, disorders that disrupt neurological processing affect ASR and PPI (Geyer, Swerdlow, Mansbach, & Braff, 1990; Braff, Swerdlow, & Geyer, 1999). Second, the brain regions that underlie the startle response are commonly affected in brain injury (Davis, 1986). Third, attentional difficulties are the most common complaint of traumatic brain-injured individuals (NIH, 2001). Finally, knowledge of how enrichment affects ASR and PPI is important to assess how specific aspects of the environment affect recovery of basic learning processes following brain injury.

Disorders that disrupt neurological processing affect ASR and PPI (Geyer, Swerdlow, Mansbach, & Braff, 1990; Braff, Swerdlow, & Geyer, 1999). PPI is particularly sensitive to neurological dysfunction, making PPI a useful way to measure changes in cognitive functioning following neurological injury or in response

to behavioral, environmental, or pharmacological manipulations intended to affect recovery.

A few studies have examined effects of brain lesions on PPI or startle habituation. Following traumatic brain injury (TBI), the reactivity of rats to acoustic and tactile startle was severely reduced compared to sham-injured rats (Hickey, Akino, Strausbaugh, & De Courten-Meyers, 1996). These results suggest that ASR is altered by traumatic brain injury and that startle procedures may provide a valuable means to assess disruptions in sensory information processing following TBI. The model of traumatic brain injury used in this study (via fluid percussion) affects brain regions known to modulate ASR and PPI, specifically the brainstem and the hippocampus.

The neural pathways underlying the startle response are located largely in the brainstem and include the ventral cochlear nucleus, the ventral nucleus of the lateral lemniscus, the nucleus reticularis pontis caudalis, and motor neurons in the facial motor nucleus and spinal cord. In rats, startle responses can be elicited electrically from each of these areas (Davis, 1984). Although this circuit is contained principally in the brainstem, other non-brainstem structures can modulate startle responses, including the hippocampus, septum, periacqueductal gray, median raphe, and inferior colliculus (Coover & Levine, 1972; Blair, Liran, Cytryniak, Shizgal, & Amit, 1978; Davis, 1984). Because these structures also are subject to brain damage resulting from closed head injury, it is likely that the startle reflex will be altered by fluid percussion injury. Whether environmental enrichment alters startle parameters

in brain-injured animals and whether these effects differ in males and females has not been examined.

Passive Avoidance

Shuttlebox passive-avoidance (often referred to as inhibitory avoidance) is an index of simple working memory in animals (Decker, 1995). In the passive avoidance task, the animal (rat) must hold information in active stores for a short period of time while that information is being acted upon, and at the same time inhibit a naturally-occurring drive. Specifically, rats must learn to remain in a lit chamber, despite having access to a preferred dark chamber, in order to avoid foot shock. Success in the passive avoidance task requires that the animal process contextual information (light-no shock, dark-shock) and learn to discriminate between a safe environment (lit side) and the unsafe side (dark side).

The effects of enrichment on passive avoidance *per se* have not been examined. However, Woodcock and Richardson (2000) examined the effect of environmental enrichment on conditioned freezing to contextual cues in Sprague-Dawley rats and found that enriched rats appeared to process contextual information faster than their standard-reared counterparts and were better able to discriminate between a conditioning context and a similar but distinctive context.

The value of passive avoidance as a measure of simple memory has specific relevance to studies examining brain injury recovery. Passive avoidance is believed to index working memory and working memory is reportedly affected by traumatic brain injury. Further, experimentally-induced brain injury has been reported to

impair passive avoidance performance in rats. Following fluid-percussion injury, lesioned animals performed worse than sham-operated controls as evidenced by significantly lower entry latencies on the test day (Hogg, Moser, & Sanger, 1998). Inclusion of the passive avoidance procedure in the present research was important to understand how enrichment might affect the recovery of simple working memory processes following brain injury.

Morris Water Maze

The Morris water maze (MWM) indexes spatial and working memory. The MWM has been used extensively to investigate the effects of various drugs on performance, age-related cognitive deficits, and the recovery of complex cognitive functions following brain injury. The MWM also has been used extensively in environmental enrichment paradigms and is thought to provide the most sensitive measure of changes in brain function in response to environmental enrichment (Rosenzweig & Bennett, 1996). Studies have consistently found environmental enrichment to enhance spatial and working memory on this task (Rosenzweig & Bennett, 1996; Pham, Ickes, & Albeck, 1999; Williams *et al.,* 2001). PESE rats learned the task more quickly, resulting in shorter latencies to reach the platform over the course of training, than did N-PESE rats (Pham *et al.,* 1999). Mice housed in a PESE environment exhibited enhanced performance on spatial learning as evidenced by shorter latencies to reach the hidden platform relative to a group of SE animals (Wiliams *et al.,* 2001). In each of these studies, PESE, SE, and N-PESE animals did not differ significantly in swim speed or swimming posture, suggesting

that performance differences could not be attributed to motor deficits. Previous studies have not examined the effects of PE alone on recovery from brain injury.

The MWM also has been used to evaluate effects of behavioral, pharmacological, and neurosurgical interventions following brain injury (Hooge & De Deyn, 2001). The Morris water maze is valuable in studies of brain injury for several reasons. First, the Morris water maze purports to test memory and spatial navigation functions. Deficits in spatial navigation and memory abilities are common after brain injury and may persist for months after injury (Passineau *et al.,* 2001). Second, performance on the Morris water maze can be repeated across several days, allowing the analysis of performance at various levels of recovery. Third, the hippocampus and overlying cerebral cortex are thought to be the primary brain regions that underlie performance on the Morris water maze. Damage to the hippocampus and cerebral cortex are the most common areas affected following brain injury.

Relevant to this study, the MWM has been used to evaluate the effects of PESE and SE on brain injury recovery. The majority of these studies have provided support for the positive effects of environmental enrichment on recovery of spatial function following brain injury. Specifically, PESE housing has been found to enhance MWM performance following anoxic injury, fluid percussion injury, and cerebral artery ligation (Passineau *et al.,* 2001; Kolb & Gibb, 1991; Ohlsson & Johansson, 1995). MWM deficits in rats with large unilateral or bilateral frontal cortical lesions were attenuated following exposure to a PESE environment (Kolb & Gibb, 1991). Injured animals recovering in PESE environments demonstrated

shorter latencies to find the platform in the MWM task than did injured animals recovering in the N-PESE environment (Passineau *et al.,* 2001). The majority of these studies have used male rats. Whether enrichment affects brain-injured males' and females' cognitive performance differently has not been examined.

Rationale for Independent Variable: Gender

Male and female brains differ in their structure and morphology and respond differently to neurological injury (Kolb *et al.,* 1998). Animal models and clinical reports suggest that females experience more recovery of brain function following injury than do males (Kolb *et al*., 1998; Grosswasser, Cohen, & Keren, 1998). Possible explanations for these differences range from pre-existing differences in cerebral lateralization and structural differences in cerebral organization (Stein, 2001) to the presence and actions of specific hormones at the time of injury (Roof & Hall, 2000). Enrichment reportedly affects recovery of cognitive function, but the extent to which these effects differ in males and females has not been examined.

The purpose of Experiment I was to evaluate the separate effects of social and physical enrichment on cognitive performance in male and female intact animals. Experiment II was designed to extend these findings to a clinically relevant problem – recovery from brain injury.

.

HYPOTHESES

This doctoral dissertation examined the effects of environmental enrichment on cognitive performance in intact male and female rats and on brain injury recovery in male and female rats. Experiment I was conducted as a 2 (male or female) x 2 (social enrichment [SE and PESE] or no social enrichment [NPESE and PE]) x 2 (physical enrichment [PE and PESE] or no physical enrichment [NPESE and SE]) full factorial experiment. The goals of Experiment I were to: 1) determine which components of the enriched environment have the greatest influence to enhance performance in neurologically-intact animals; and 2) determine whether males and females differ in their response to environmental enrichment across various cognitive measures. Experiment II was conducted as a 2 (male or female) x 2 (social enrichment [SE and PESE] or no social enrichment [NPESE and PE]) x 2 (physical enrichment [PE and PESE] or no physical enrichment [NPESE and SE]) full factorial experiment. The goals of Experiment II were to: 1) determine which components of the enriched environment have the greatest influence on recovery of cognitive functioning following brain injury; 2) determine whether males and females differ in their recovery from brain injury across various measures; and 3) determine whether brain-injured males and females differ in their response to environmental enrichment across various cognitive measures.

There were four major hypotheses for Experiment I and four major hypotheses for Experiment II. Social enrichment refers to housing rats in groups to provide opportunities for social interaction. Physical enrichment refers to providing toys and objects to provide opportunities for physical stimulation. Most studies of

enriched environments provide both physical enrichment (PE) and social enrichment (SE) and, therefore, are labeled as “PESE” in this report. Isolation rearing refers to housing animals individually without toys. The isolation environment, that does not provide physical enrichment or social enrichment, is referred to as “NPESE” in this report. In Experiment I, enhancement of performance was assessed by comparing the performance of animals in each housing condition. In Experiment II, the degree of recovery was assessed by comparing performance of animals in each of the housing conditions following brain injury.

Hypotheses: Experiment I

Hypothesis 1

Environmentally enriched (PE, SE, or PESE) animals will exhibit superior performance on all tasks and measures (*i.e.*, increase habituation in open-field activity, increase habituation in ASR, increase PPI, enhance passive avoidance performance, enhance Morris water maze performance) when compared to non-enriched (isolated) animals.

Rationale

Rats reared in a combined physically- and socially-enriched environment exhibit enhanced learning compared with socially-reared and isolation-reared rats (Gardner, Boitano, Mancino, & D' Amico, 1975; Smith, 1972; Varty *et al*., 2000).

Hypothesis 2

Performance on simpler measures of cognitive functioning (*i.e.*, open-field activity, acoustic startle activity, pre-pulse inhibition) will be associated with performance on more complex measures (*i.e.*, Morris water maze).

Rationale

The Morris water maze is a spatial memory task. Memory is dependent on attention and information processing. Success on this task requires intact information processing and intact attention. Animals that exhibit deficits in attention and simple information processing also will exhibit impairments on this task.

Hypothesis 3

For male subjects, the effects of enrichment to enhance cognitive performance will be: PESE > SE = PE > N-PESE.

Rationale

Previous studies with male rats have reported that the combined enriched environment (PESE) has the greatest effect to enhance cognitive performance (Gardner, Boitano, Mancino, & D' Amico, 1975; Smith, 1972; Varty *et al*., 2000).

Hypothesis 4

For female subjects, the effects of enrichment to enhance cognitive performance will be: SE $\geq$ PESE > PE = NPESE.

Rationale

Pilot work that compared the effects of social and physical enrichment on cognitive measures in intact females obtained these results.

Hypotheses: Experiment II

Hypothesis 1

Environmentally enriched (PE, SE, or PESE) animals will exhibit superior performance on all tasks and measures (*i.e.*, increase habituation in open-field activity, increase habituation in ASR, increase PPI, enhance passive avoidance performance, enhance Morris water maze performance) when compared to non-enriched (isolated) animals.

Rationale

Brain-injured rats recovering in enriched environments exhibit enhanced cognitive performance compared with brain-injured rats recovering in isolation or rats

recovering in socially-enriched conditions (Passineau *et al*., 2001; Ohlsson & Johansson, 1995).

Hypothesis 2

Performance on simpler measures of cognitive functioning (*i.e.,* open-field activity, acoustic startle activity, pre-pulse inhibition) will be associated with performance on more complex measures (*i.e.,* Morris water maze).

Rationale

The Morris water maze is a spatial memory task. Memory is dependent on attention and information processing. Success on this task requires intact information processing and intact attention. Animals that exhibit deficits in attention and simple information-processing also will exhibit impairments on this task.

Hypothesis 3

For male subjects, the effects of enrichment to enhance cognitive performance will be: PESE > SE = PE > N-PESE.

Rationale

Studies of brain-injured rats recovering in enriched environments report that the combined enriched environment (PESE) has the greatest effect to enhance cognitive performance (Passineau *et al.,* 2001; Ohlsson & Johansson, 1995).

Hypothesis 4

For female subjects, the effects of enrichment to enhance cognitive performance will be: SE $\geq$ PESE > PE = NPESE.

Rationale

Pilot work that compared the effects of social and physical enrichment on cognitive measures in intact females obtained these results.

METHODS

Overview

This doctoral dissertation research project examined the separate effects of social and physical enrichment on cognitive performance in neurologically intact and brain-injured male and female Sprague-Dawley rats. Experiment I was run using intact animals. Experiment II was run using brain injured animals.

Experimental Design and Determination of Sample Size

Experiment I was conducted as a 2 (male or female) x 2 (physical enrichment [PE and PESE] or no physical enrichment [NPESE and SE]) x 2 (social enrichment [SE and PESE] or no social enrichment [NPESE or PE]) full factorial design with 24 subjects per cell. Experiment II was conducted as a 2 (male or female) x 2 (physical enrichment [PE and PESE] or no physical enrichment [NPESE and SE]) x 2 (social enrichment [SE and PESE]) or no social enrichment [NPESE or PE]) full factorial design with 12 subjects per cell. These sample sizes were selected to optimize statistical power across a range of dependent measures that vary in effect size in response to environmental enrichment and brain injury (*e.g.*, Passineau et al., 2001; Van Praag *et al*., 1999).

Sample size determination analyses were conducted using the procedures of Keppel (1991); Keppel, Saufley, and Tokunaga (1992); and Cohen (1988). Estimates of effect size in the population were determined by calculating an estimated omega squared (ϖ^2) according to the formula:

$$\varpi^2_A = \frac{\sigma^2_A}{(\sigma^2_A + \sigma^2_{S/A})}$$

where σ^2_A refers to the estimated population treatment effects and $\sigma^2_{S/A}$ refers to the estimated population error variance (Keppel *et al*., 1992, p. 180). The omega squared statistic provides measure of effect size that is relatively independent of sample size and is expressed as a proportion of the total variability ($\sigma^2_A + \sigma^2_{S/A}$) that is associated with the treatment or manipulation (σ^2_A).

These calculations revealed that to detect an effect of enrichment 24 subjects per cell were necessary in neurologically intact animals and 9 subjects (*e.g*., Passineau *et al*., 2001 used 7 animals/cell and obtained an effect size of 0.8 using the above calculations) were necessary in brain-injured animals. One additional animal was then added to each cell in Experiment II to allow for the potential loss of subjects in response to brain injury. The addition of one animal to each treatment cell was based on an anticipated morbidity rate of 10% in animals following the level of injury to be used in this experiment (Ling & Garcia-Pinto, 1999). Further, in Experiment II, two additional animals were added to each cell to create equal social groups of 3 animals each in the PESE and SE conditions.

Research Design and Methods Relevant to Experiment I

Subjects

The subjects in Experiment I were 192 (96 male and 96 female) adult (43-45 days old) Sprague-Dawley rats (Charles River Laboratories).

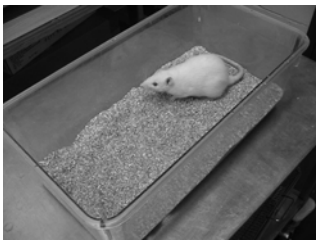

Figure 1. NPESE

Housing. See Figures 1-4

Figure 2. PE

All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at 23^{o} C and 50% relative humidity on a 12-hour reversed light/dark cycle (lights on at 1600 hours). Animals were assigned to one of four housing conditions (PESE, SE, N-PESE, or PE). In housing condition N-PESE, animals were single-housed in standard polycarbonate rat cages (40 cm x 20 cm x 20 cm). In condition PE, animals were single-housed in standard rat cages (40 cm x 20 cm x 20 cm) and a variety of toys (durable dog and cat toys; *e.g.,* colored textured balls, rings, and bones) were placed in the cage to provide physical and tactile stimulation. Objects were removed 2-3x/week (or sooner if damaged) and were replaced with new objects. The objects used, changing schedule, and cage dimensions were based on methods described in previous studies (Varty *et al.,* 2000; Gardner *et al.,* 1975). In condition SE, animals were housed in groups of three in large rat cages

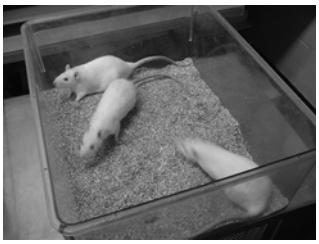

Figure 3. SE

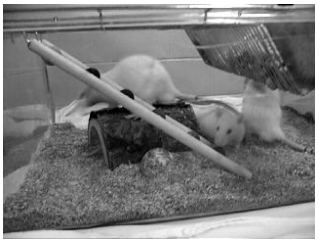

Figure 4. PESE

(46 cm x 36 cm x 20 cm). In condition PESE, animals were housed in groups of three in large rat cages (46 cm x 36 cm x 20 cm) and were provided toys according to the procedures described above. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Pub, 82-23, rev. 1985).

Procedure

For logistical purposes, subjects were run in balanced cohorts of 48 (see Table 1 for experimental timeline/cohort). During an 8-day Baseline Phase, subjects were acclimated to the facility and to the equipment. During this phase, animals were housed individually in standard polycarbonate shoebox cages (42 x 20 x 20 cm). On day 1, animals arrived at the facility. On days 2-3, animals were handled once a day for 5 minutes. Handling reduces the stress associated with repeated handling that is necessary to conduct behavioral measures. All animals then underwent three acclimation exposures to the startle and pre-pulse procedures (days 4, 5, and 8) and one acclimation to the locomotor (open field) chambers (day 6). Acclimation was done to minimize the contamination of responses by the stressful effects of exposure to a novel situation (Faraday & Grunberg, 2000). Acclimation procedures do not affect later measurement of habituation. On day 7, baseline open field (OF) activity was measured and on day 9 baseline ASR amplitude was measured. Baseline data for these measures were used to balance experimental groups. The experimental time line used during the

acclimation/baseline period was based on previous studies in this laboratory in which these behavioral measures were used (Faraday *et al*., 1999a; 1999b; Faraday & Grunberg, 2000).

On the 10th day post arrival, animals were assigned to one of four housing conditions (PESE, SE, N-PESE, or PE). Animals remained in the experimental housing conditions for an additional 11 days (Enrichment Period: experimental days 11-21, Table 1) prior to behavioral testing and throughout the testing period. This procedure was used to parallel the procedures in Experiment II in which animals were allowed an 11-day post-injury recovery period before behavioral testing began.

Table 1. Experiment I: Experimental Timeline

Phase	Procedures
Day 1	Animals Arrive
Day 2-3	Gentling
Day 4	ASR-PPI Acclim 1
Day 5	ASR-PPI Acclim 2
Day 6	OF acclimation
Day 7	Baseline OF test
Day 8	ASR-PPI Acclim3
Day 9	ASR-PPI Baseline
Day 10	Assign to groups
Day 11-21	Enrichment Period
Day 22	Locomotion
Day 25	ASR and PPI
Day 27	Locomotion
Day 30	ASR and PPI
Day 32	MWM Day 1
Day 33	MWM Day 2
Day 34	MWM Day 3
Day 35	MWM Day 4
Day 36	MWM Day 5
Day 38	Locomotion
Day 40	ASR and PPI

Dependent Variables

Open Field

Open field activity was measured on days 22, 27, and 38 (enrichment days: 12, 17, and 28). This testing schedule brackets the period in which enriched environmental effects were found in rats on this measure (Varty *et al.,* 2000). Open field activity was measured using an Omnitech Electronics Digiscan infrared photocell system (Test box model RXYZCM [16 TAO]; Omnitech Electronics, Columbus, OH).

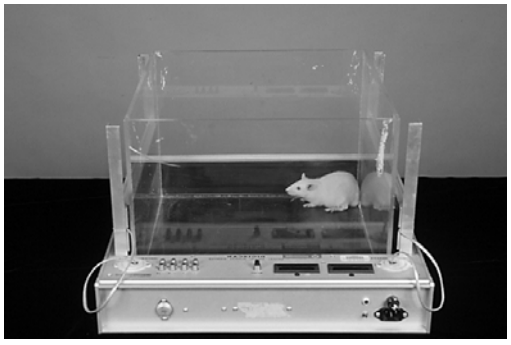

Figure 5. Locomotion Chamber

Animals were placed singly in a 40 x 40 x 30 cm clear Plexiglas arena and a Plexiglas lid with multiple 3.5 cm diameter holes was placed on top of the arena. The lid ensures that subjects have adequate ventilation but cannot escape during data collection. A photocell array measured horizontal activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2 cm above the arena floor. A second side-to-side array of 16 pairs of additional photocells located 10.5 cm above the arena floor measured vertical activity. Data were transmitted to a computer via an Onmitech Model DCM-I-BBU analyzer. Once subjects were placed in the test arenas, the experimenter turned off the lights and left the room. The apparatus monitored animal activity continuously for a total testing period of 1 hour.

The interfaced software generates 21 subvariables, including total distance in cm (a measure of overall activity) and horizontal and vertical activity (measures of activity in the horizontal plane and exploratory activity, respectively). Horizontal activity was analyzed as the primary index of general activity and exploration. Groups that exhibit the lowest activity levels during the 1-hour testing session were interpreted as exhibiting the greatest habituation.

Acoustic startle reflex (ASR) with and without pre-pulse inhibition (PPI)

Figure 6. Acoustic Startle (ASR)

Acoustic startle reflex amplitudes and pre-pulse were measured in an Acoustic Response Test System (MED-ASR-310; Med Associates, Georgia, VT) consisting of weight-sensitive platforms inside individual sound-attenuated chambers with speakers, an audio generator, a high-speed serial microcontroller, and a high-speed analog to digital converter. Responses were measured on experimental days 25, 30, and 40 (enrichment days 15, 20, and 30) (See Table 1, p. 39). Each rat was placed individually in a ventilated holding cage. The holding cages were small enough to restrict extensive locomotion but large enough to allow the subject to turn around and make other small movements. Each cage was placed on a weight-sensitive platform. Test sessions lasted approximately 24 minutes and consisted of initial and final blocks of 3 pulse trials (120 db), separated by one block that included 8 pulse only trials and 10 of each of the pre-pulse trials (75 dB, 82 dB, or light). A light stimulus was included in order to examine sensory-gating responses to visual as well as auditory stimuli. These procedures and stimuli are widely used and allow for the measurement of both sensory gating and startle habituation (Farid, Martinez, Geyer, & Swerdlow, 2000; Swerdlow, Braff, & Geyer, 2001).

Startle habituation was determined based on changes in startle magnitude from the initial to the final block of pulse only trials. Habituation was calculated as [(average startle amplitude during Block 3)/(average startle amplitude during Block

1)] x 100. Percent pre-pulse inhibition was calculated as [(amplitude of trial without pre-pulse) - (amplitude of trial with pre-pulse)/amplitude of trial without pre-pulse] x 100. These calculations are standard (Faraday & Grunberg, 2000; Swerdlow, Braff, & Geyer, 2001; Acri, 1994).

Passive Avoidance

After all other dependent measures were gathered, 48 neurologically-intact females assigned to N-PESE, PE, SE, or PESE housing conditions were tested in a passive avoidance task (ED 43-44) to determine whether this measure of simple memory would be useful in future experiments. Animals were trained and tested using an automated avoidance training system (Gemini, San Diego Instruments, San Diego, CA) consisting of two 21 x 25 x 17 cm chambers separated by a vertically-sliding door. Lighting in the chambers was provided by a 50-watt bulb 3 cm above the translucent ceiling.

Figure 7. Passive Avoidance (PA)

Scrambled, constant-current shocks were delivered through a grid floor. Control of the door, lighting, and shock was provided by means of a 486-personal computer running propriety software (PA, San Diego Instruments, San Diego, CA).

Training and testing procedures were similar. During training, the animal was placed in one chamber of the darkened apparatus. After a delay of 60 seconds, a light came on and the door to the other, still darkened chamber, opened. Naive rats generally move from the lit chamber into the dark chamber in less than 60 seconds. When the rat crossed completely into the darkened chamber, the door closed, latency to cross was recorded by the interfaced computer, and a 0.4 mA shock was

delivered through the grid floor for 1 sec. The rat was left in the darkened chamber in which the shock had been delivered for 30 seconds and then was removed. If the rat did not cross into the darkened chamber, then it was removed after 300 seconds.

The testing procedure was identical except that shock was not delivered if the animal crossed into the darkened chamber. Memory was presumed to have occurred if the animal did not cross into the chamber in which it previously was shocked, or if latency to cross was statistically significantly longer during the testing trial than during the training trial. In Experiment II, testing was carried out 24 hours after training on experimental day 44 (enrichment day 33).

Morris Water Maze

Morris Water Maze was performed on experimental days 32-36 (enrichment days 22-26). The water maze consists of a circular, dark blue plastic tank 96 cm in diameter (72 cm in diameter at water level) and 50 cm high filled with 25^{o} C (±1^{o}C) water. The platform is a black acrylic structure that is 1.5 cm below the water surface. To ensure that the rats cannot see the platform, black nontoxic water-soluble Tempera paint was added to the water. Maze sessions were recorded using a computerized video tracking system (Polytrack System, San Diego, CA). Latency to find the platform and total path length were analyzed to index memory and learning. The task used in this study is used widely in the

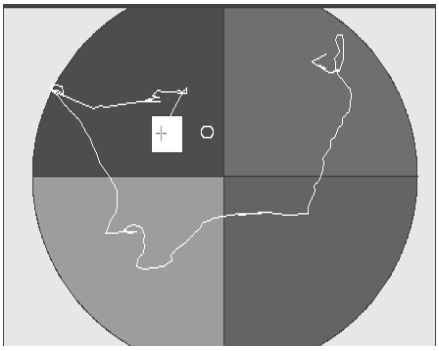

Figure 8. Morris Watermaze Tracking

enrichment literature (Daniel et al., 1999; Passineau et al., 2001; Pham et al., 1999; Jenkins et al., 1999).

The platform was hidden beneath the water surface in the northeast maze corner. The platform remained in a fixed position for all trials. Each animal received 4 trials per day for 5 days for a total of 20 trials; with a maximum trial swimming time of 1 min (animals that did not climb on the platform after 1 min were guided to it). Animals were allowed to remain on the platform for 30 sec. Then, animals were removed for a 3-minute inter-trial interval. The starting locations for each trial were randomized (North, South, West, or East) with no two consecutive trials starting from the same location.

Research Design and Methods Relevant to Experiment II

Subjects

Subjects were 96 (48 males and 48 females) adult (43-45 day old) Sprague-Dawley rats, resulting in 12 subjects per cell.

Housing

All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at 23^0 C and 50% relative humidity on a 12-hour reversed light/dark cycle (lights on at 1600 hours). Animals were assigned to one of four housing conditions (PESE, SE, N-PESE, or PE) as described in Experiment I. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Pub, 82-23, rev. 1985).

Procedures

The procedures in Experiment II were the same as in Experiment I except that animals in Experiment II underwent brain injury on day 10. To avoid confounding motor deficits with cognitive deficits, testing began 11 days post injury. Previous studies of traumatic brain injury (TBI) have found that motor deficits generally recover in 11 days (Ling & Garcia-Pinto, 1999; Passineau *et al.,* 2001).

Brain Injury Model: Fluid Percussion Injury

Table 2. Experiment II: Experimental Timeline	
Phase	Procedures
Day 1	Animals Arrive
Day 2-3	Gentling
Day 4	ASR-PPI Acclim 1
Day 5	ASR-PPI Acclim 2
Day 6	OF acclimation
Day 7	Baseline OF test
Day 8	ASR-PPI Acclim3
Day 9	ASR-PPI Baseline
Day 10	Assign to groups Brain Injury/neuroscore
Day 11-21	Enrichment Period
Day 22	Locomotion
Day 25	ASR and PPI
Day 27	Locomotion
Day 30	ASR and PPI
Day 32	MWM Day 1
Day 33	MWM Day 2
Day 34	MWM Day 3
Day 35	MWM Day 4
Day 36	MWM Day 5
Day 38	Locomotion
Day 40	ASR and PPI
Day 43	Passive avoidance-train
Day 44	Passive avoidance-test

Human traumatic brain injury is heterogeneous. No model, therefore, can reproduce all that occurs within a given clinical traumatic brain injury. The fluid percussion (FP) model, however, has been shown to reproduce many of the critical aspects of TBI including the behavioral and neuropathological sequelae commonly found in human traumatic brain injury (Mattiasson *et al.,* 2000). During a closed head trauma, the injury process is initiated by the impact of the brain against the inner surface of the skull (Ling & Garcia-Pinto, 1999). Following impact, there is an immediate and complex biochemical, cellular, and physiological injury cascade that culminates in the neurological dysfunction observed in head injured individuals.

The fluid percussion model is an impact-induced brain trauma model that replicates a closed or non-penetrating head injury. Instead of the brain striking the inside of the skull, a fluid column creates the impact that causes the injury. A pendulum arm strikes a piston at the end of a fluid-filled reservoir (Ling & Garcia-Pinto, 1999). The contact between the pendulum arm and the piston creates a force wave that propagates through the fluid-filled tubing to the brain surface, beginning the injury cascade.

The fluid percussion model of brain injury was used in this research because it is the most widely used model of traumatic brain injury in rats (TBI) and because it has produced the most reliable and consistent results (Ling & Garcia-Pinto, 1999). The procedures used in this study were based on those used by other investigators in studies of brain injury and environmental enrichment (Passineau *et al*., 2001; Ling & Garcia-Pinto, 1999). Prior to surgery, rats were anesthetized with halothane using an induction chamber. Anesthesia was maintained during surgery using a face mask. Then, animals were prepared for surgery using procedures described in the literature (Passineau *et al*., 2001; Ling & Garcia-Pinto, 1999). The surgical procedure was used to insert the cannula necessary to deliver the fluid percussion injury.

Figure 9. Fluid Percussion (FP) Device

Prior to surgery, all animals underwent the neuroscore test. The neuroscore test is a well-established test designed to validate severity of injury following fluid percussion (Dixon *et al*., 1987). Testing occurs prior to injury to obtain baseline data and immediately following injury to validate injury. Results from the neuroscore tests correlate with the severity of the damage following the fluid percussion injury (Dixon *et al*., 1987; McIntosh, *et al*., 1989). The neuroscore is a composite score of neuromotor function where the maximum score is 20 points. The fluid percussion injury typically generates motor impairments in the region of the body contralateral to the site of injury, resulting in weakness and discoordination. Following the injury,

motor abilities are tested and scored by the examiner. Scoring for the animals ranges from 0 (severely impaired) to 4 (normal) on each of the following indices: right and left forelimb flexion, resistance of lateral pulsion to the left and right, ability to stand on an inclined plane in angles up to 40^{o}, exploratory behavior (Dixon *et al.,* 1987).

In preparation for surgery, each subject was placed prone on a flat surface and its head was constrained in a stereotaxic head frame. Then, a 1.5 cm sagittal incision was made from the midpoint between the ears towards the nose with a scalpel and the overlying skin and muscle were reflected to expose the cranium. Next, the position for the burr hole (point of impact) at 2.5 mm lateral to the central skull suture and 3.8 mm posterior to the bregma skull suture was measured and marked with a non-toxic marker. Then, a hole was drilled into the surface of the skull using an electric drill with a 1-mm round-head drill bit. Next, a head cannula was introduced through the hole until it abutted the dural surface of the brain and was affixed to the skull using cyanoacrylate glue. Following surgery, brain injury was induced in each animal using the fluid percussion method.

To prepare for injury, the head cannula was filled with saline and the tubing from the percussion device was filled with water. The tubing was attached to the head cannula. Then, injury was induced by releasing the pendulum arm of the fluid percussion instrument and allowing it to fall freely so that it strikes the piston. An oscilloscope was used to ensure that the proper force was applied to the skull. For this study, trauma was delivered at 2.5 atm, which is considered a moderate-severe injury (Passineau *et al.,* 2001). Histopathological effects associated with this level

and type of injury include damage to the ipsilateral hippocampus, cerebral cortex (frontoparietal cortex) and white matter tracts (external capsule) (Passineau *et al.,* 2001; McIntosh *et al.,* 1989). Behavioral and cognitive effects occur with this level of injury (Hamm, Lyeth, & Jenkins *et al.,* 1993).

DATA ANALYTIC STRATEGY

The goals of data analyses were to determine the extent to which physical and social enrichment separately and together affected recovery of cognitive performance following brain injury and the extent to which the magnitude of enrichment effects varied as a function of sex. For all animals, open-field (OF), acoustic startle response (ASR), pre-pulse inhibition (PPI), and Morris water maze (MWM) responses were analyzed with separate repeated-measures analyses of variance (ANOVA) with Sex, Physical Enrichment, and Social Enrichment as the between-subject factors and Time as the within-subjects factor. If there were significant between-subject effects, then univariate ANOVAs on each day were performed. Interactions were examined using separate ANOVAs following the procedures of Keppel (1991). To the extent possible, experimental groups were balanced for ASR, pre-pulse inhibition, and locomotion at the conclusion of the baseline period. Despite this strategy, baseline differences existed on measures of PPI, so baseline responses were used as covariates in the analyses of % PPI data. Passive avoidance data were evaluated using paired-t tests to compare performance on the training and testing day and determine if learning occurred (latencies increased from training to testing day). Then, univariate analyses were performed on the training and testing days as described above. There were no

group differences in latency to cross on the training day; therefore, training day values were not used as covariates in the analyses of testing day performance.

Because passive avoidance latency data and Morris water maze data did not consistently meet criteria for parametric statistics (*i.e.,* normal distribution, homogeneity of variance), these data also were analyzed using nonparametric ANOVAS (*i.e.*, Kruskal-Wallis test). Water maze data also were analyzed in a binary format with data coded based on a median split of mean performance times. These data were analyzed using chi-squares. Passive avoidance also were analyzed in a binary format with data coded in terms of whether or not the animal successfully performed the task (did not cross into the darkened chamber). These data also were analyzed using chi-squares.

Values of eta-squared were used to determine the relative magnitude of enrichment effects for each group. Eta squared is a measure of effect size that indicates the proportion of variance explained by a given independent variable. In analysis of variance terms, it is the ratio of the between-groups sum of squares to the total sum of squares (Cohen & Cohen, 1983).

All tests were two-tailed with $p < 0.05$. In the current study, several dependent variables were used and therefore several statistical tests were run. Because multiple analyses were conducted, several strategies were employed to minimize the probability of Type 1 error. First, the experiments were designed to provide adequate power (*i.e.,* 0.80). When sample size supports adequate power, the likelihood of Type I errors is minimized. Second, global analyses incorporating all factors (Physical enrichment, Social enrichment, Sex) were used to guide internal

analyses. Sub-group analyses followed only if overall analyses revealed significant main effects or interactions. This strict Fisherian strategy is consistent with recommendations of Keppel (1991) and Cohen and Cohen (1983) and substantially reduces the number of tests performed. Finally, the error term (the within-subjects variance that constitutes the denominator of the F ratio) specific to the comparison being made was used rather than the error term for all subjects. This technique controls Type I error because as the denominator degrees of freedom decrease, the F value necessary to achieve significance for a given comparison increases.

RESULTS

Summary of Results Presentation

In this research the separate effects of social and physical enrichment on cognitive processes were examined to determine which aspects of enrichment had the greatest effect to alter cognitive performance in rats. For the purposes of this study, the “social” effect is based on the combined groups of SE and PESE, unless otherwise specified. Similarly, the “physical” effect is based on the combined groups of PE and PESE unless otherwise specified. Results from the specific experimental groups (NPESE, PE, SE, or PESE) are presented only when needed for clarification.

The current study included four experimental conditions, four tasks, and multiple trials for each dependent variable. Both basic (locomotor habituation, ASR, and PPI) and complex measures (Morris water maze and passive avoidance) of cognition were included to determine if enrichment affected performance at varying levels of complexity. Results from Experiment I and Experiment II are presented separately. First, all of the data from Experiment I are presented. Next, data are

presented which compare the performance of non-injured and injured animals from selected behavioral measures to verify injury. Finally, data from Experiment II (injured animals) are presented.

For each experiment, results are presented in order of increasing task complexity. Locomotion data that index simple information processing and represent the most basic level of cognitive performance are presented first. ASR and PPI represent general arousal and attentional functions and are used to index a slightly higher level of cognitive performance. The ASR/PPI data are presented second. Passive avoidance is a simple working-memory task that requires the ability to process information (*i.e.,* black box = shock; white box = no shock) and remember the information when it is presented later (*i.e.,* testing day). Passive avoidance is a more complex task than either locomotor activity or ASR and PPI. Passive avoidance data are presented third. Finally, Morris water maze, a task that requires the animal to utilize a variety of cognitive skills (*e.g.,* information processing, attention, and spatial memory), represents the highest level of cognitive performance. Water maze data are presented last. Tables containing F values, degrees of freedom, and p values for Experiment I are presented in Appendix A. Tables containing F values, degrees of freedom, and p values for data comparing injured and intact animals are presented in Appendix B. Tables containing F values, degrees of freedom, and p values for Experiment II are presented in Appendix C.

Experiment I. Intact Animals

Locomotion

Horizontal activity in the open field reflects overall activity level and can be used as an index of simple information-processing. When initially placed in the activity chamber, animals' activity levels are high. As the animal acclimates to the testing chamber, activity levels drop and eventually level off. Therefore, persistent high levels of activity suggest that the animal is not acclimating to the novel environment or is not processing information efficiently. In contrast, persistent low levels of activity suggest faster acclimation and more efficient processing of environmental cues. In this experiment, activity was measured for 1 hour at four separate time points (baseline, enrichment days (ED) 12, 17, and 28). Baseline levels of activity were measured prior to the enrichment period for the purpose of balancing experimental groups.

***Analytic Approach*.** Horizontal activity data were analyzed using repeated-measures analyses of variance (ANOVA) to determine whether activity levels changed across the experimental phase (baseline to ED 28) with repeated exposure to the testing chambers. Higher overall levels of activity reflect hyperactivity and decreased information processing. Lower activity levels reflect habituation and increased information processing (Varty *et al.,* 2000). At first, all animals were analyzed together. Then, because sex differences in activity were the largest differentiating variable and because the experiment was designed to examine potential gender differences in sensitivity to physical and social enrichment, the effects of physical and social enrichment were examined separately for males and

females. Because there were between-group differences and because there was an overall Time x Social interaction, univariate analyses were performed on each day. Repeated-measures ANOVAS then were performed on each day (*i.e.,* within session activity) to evaluate further the pattern of activity over the 1-hour session and to determine how quickly or slowly habituation to the testing chamber occurred. F values, degrees of freedom and p values for analyses in Experiment I are provided in Appendix A.

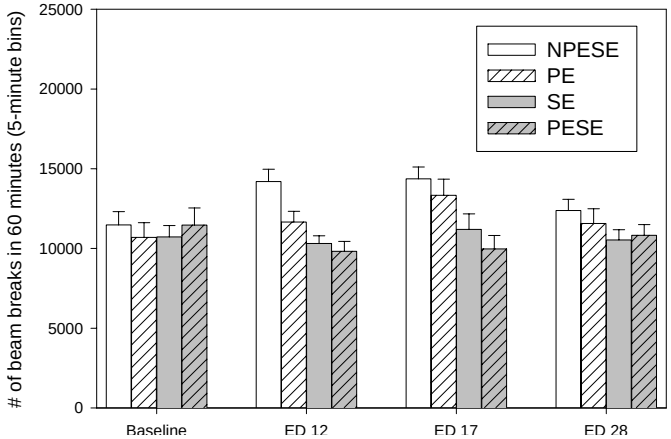

Figure 10. Intact males: horizontal activity across days

Activity Habituation. *See Figures 10-11*. First, all animals were analyzed together using repeated-measures analyses of variance over the four testing days (*i.e.,* from baseline to enrichment day (ED) 28). When all animals were analyzed together, there was no overall significant effect for Time across the multiple testing periods, but there was an overall Time X Social interaction [F (3, 486)= 2.96, $p < 0.05$], suggesting that socially-reared animals differed from isolated animals in their activity patterns across the different testing periods (Time). Females were more active than males [Gender: F (1, 162) = 23.07, $p < 0.001$] and isolated

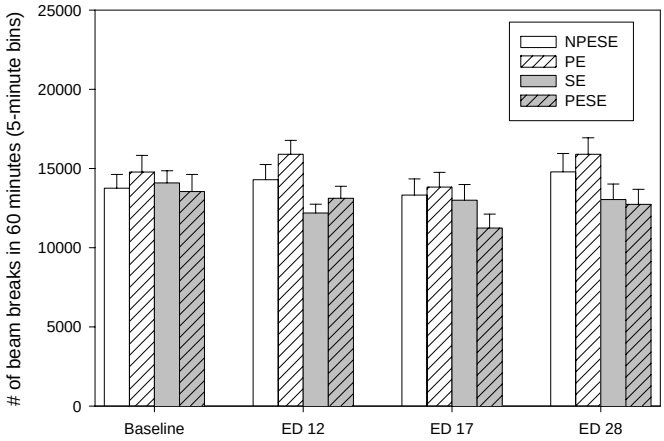

Figure 11. Intact females: horizontal activity across days

animals (NPESE and PE) were more active than socially-enriched (PESE and SE) animals [Social: F (1, 162) = 17.12, p <0.001].

Because there was a main effect for gender, the main effects of social and physical enrichment were examined separately for males and females. Within both males and females, isolated animals were more active than socially- enriched animals [M-Social: F (1, 72) = 10.50, p <0.05; F-Social (1, 90) = 7.05, p <0.05].

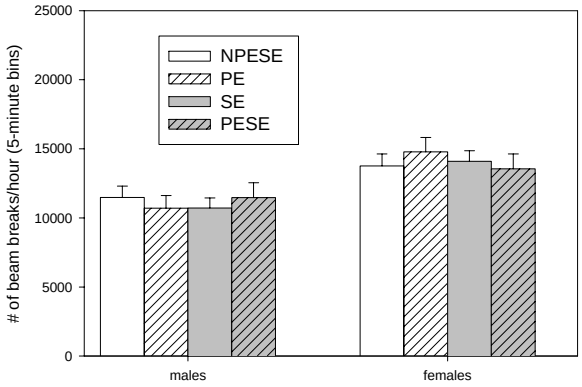

Figure 12. Intact Animals: Baseline Horizontal Activity

For males, but not for females, the effects of social enrichment also varied across the experimental period [Time X Social: F (3, 216) = 2.68, p <0.05]. Therefore, univariate analyses were conducted on each day.

Baseline activity levels. *See Figure 12.* When all animals were considered together, there were no significant group differences. Therefore, baseline activity was not used as a covariate when univariate analyses were run on each day.

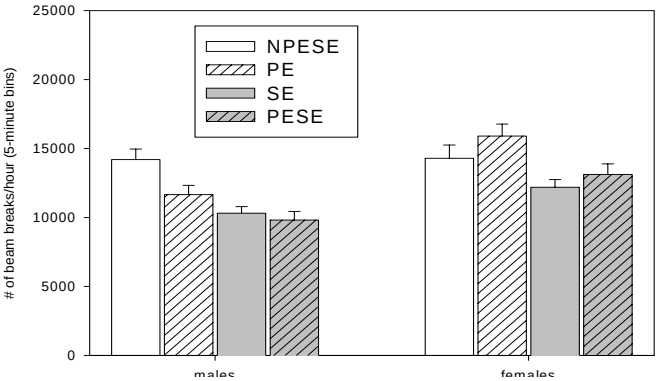

Figure 13**.** Intact Animals: Horizontal Activity ED 12

Enrichment Day 12. *See Figure 13.* Data from two animals out of a total of 192 animals (1 NPESE male; 1 PE male) could not be used because of equipment or software failure. Females were more active than males [Gender: F (1, 182) = 21.70, p < 0.001] and isolated animals were more active than socially-enriched animals [Social: F (1, 182) = 26.85, p <0.001]. The effects of physical enrichment differed in

males and females [Gender X Physical: F (1, 182) =7.42, p <0.05], such that physical enrichment decreased activity in males, but increased activity in females.

When the sexes were considered separately, group differences were present for males and females. Specifically, within males, both physically and socially-enriched animals were less active than non-physically and isolated animals respectively [Social: F (1, 92) = 19.56, p =<0.001; Physical: F (1, 92) = 5.50, p < 0.05]. For males, comparison of activity levels among the four treatment groups revealed that the PESE group had the lowest levels of activity overall. In contrast, within females, there was a main effect for social enrichment only, such that animals in the socially-enriched conditions were less active than were animals in the isolated conditions [Social: F (1, 90) = 9.42, p <0.05). There was no main effect for physical enrichment on activity for females on enrichment day 12.

Enrichment Day 17. *See Figure 14.* Data from 18 animals out of a total of 192 were not used (5 male NPESE; 5 male PE; male SE; 3 male PESE; 1 female NPESE; 1 female PE) because of equipment or software failure (computer crashed in the middle of session). When all animals were considered together, male and female activity patterns did not differ significantly. Isolated animals were more active than socially-enriched animals [Social: F (1, 168) = 12.46, p <0.001]. To parallel the previous data analytic strategy, the sexes were examined

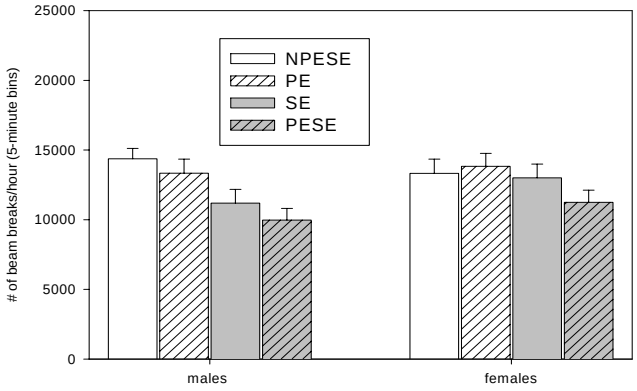

Figure 14. Intact Animals: horizontal activity ED 17

separately. A main effect for social enrichment was present only in males, with animals in the isolated groups exhibiting less activity than animals in the social groups [F (1, 76) = 12.97, p <0.001]. For males, a main effect for physical enrichment on activity levels was no longer present on this measurement day. Within females, there were no main effects of either social or physical enrichment on activity levels on Enrichment Day 17.

Enrichment Day 28. *See Figure 15.* On enrichment day 28, data from 4 animals (2 male NPESE, 2 male PE) out of a total of 192 were not used because of equipment or software failure (computer crashed in the middle of session and data were not accumulated). When all animals were considered together, females again were more active than males [Gender: F (1, 180) = 18.97, p <0.001] and animals in the isolated groups were more active than the animals in the social groups [Social: F (1, 180) = 8.59, p <0.05]. When data were analyzed within gender, the patterns of activity for males was similar to enrichment day 17 with a trend for isolated animals to exhibit greater activity than the social animals [Social: F (1, 88) = 3.08, p = 0.083]. A similar pattern was observed for females with isolated animals exhibiting more overall activity than the socially-reared animals [Social: F (1, 92) = 5.65, p <0.05]. There were no effects of Physical enrichment or Social x Physical interactions on activity patterns of males or females.

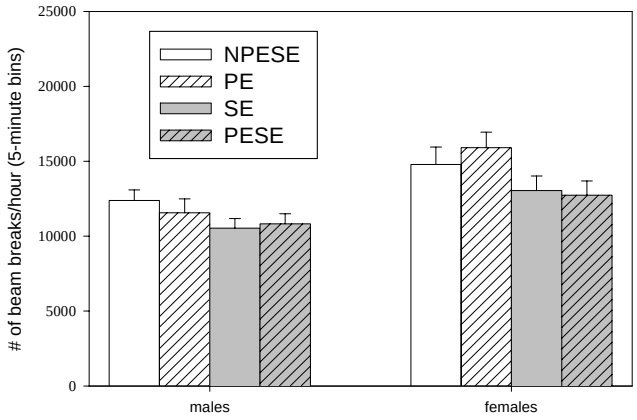

Figure 15. Intact Animals: Horizontal Activity ED 28

Horizontal Activity within Session. To further assess the effects of enrichment on each measurement day, repeated-measures ANOVAs were used to assess activity patterns within the 60-minute testing session. These analyses provided a clearer picture of how activity levels changed across the testing session.

Within-Session Analyses

Enrichment Day 12. See Figures 16-17. Because there was a significant main effect for social enrichment on each testing day, within session activity was examined for each day using repeated-measures analyses of variance (ANOVA). On enrichment day 12, when all animals were analyzed together, there was a significant effect of Time, with all groups decreasing activity across the testing session (Time: F (11, 2002) = 347.94, p<0.001]. Females were more active than males [Gender: F (1, 182) = 21.16, p <0.001] and isolated animals were more active than were socially-enriched animals [Social: F (1, 182) = 26.61, p <0.001]. When data were analyzed separately within gender, both the isolated males [Social: F (1, 92) = 18.57, p <0.001] and females [Social: F (1, 90) =

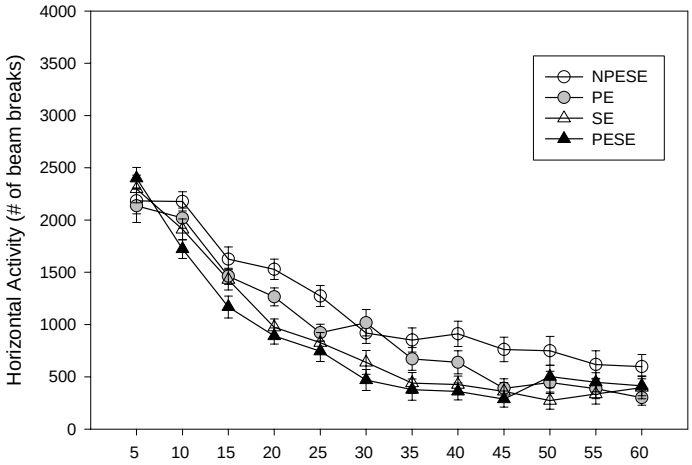

Figure 16. Intact Males: Within Session Activity ED 12

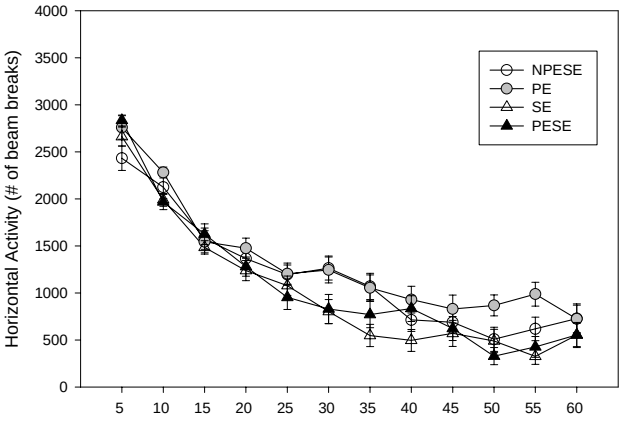

Figure 17. Intact Females: Within Session Activity ED 12

9.361, p =0.05] exhibited greater activity over time (less habituation) compared to socially-enriched animals. Within males, but not females, physical enrichment also increased habituation [Gender X Physical interaction: F (1, 182) = 6.97, p = 0.05; Physical: F (1, 92) = 5.28, p =0.05].

Enrichment Day 17. See Figures 18-19.

On day 17, when all animals were analyzed together, there was a significant effect of Time with all groups decreasing activity across the testing session [Time: F (11, 1848) =325.71, p <0.001]. Isolated animals exhibited higher levels of activity compared to socially-enriched animals [Social: F (1, 168) = 12.46, p <0.001]. When data were analyzed separately within gender, males exhibited significantly decreasing activity across the 60-minute testing period and these effects varied depending on housing condition [Time: F (11, 836) = 139.98, p <0.001; Time X Social: F (11, 836) = 2.93, p <0.001]. Isolated males [Social: F (1, 76) = 12.97, p < 0.001] exhibited more activity over time (less habituation) compared to socially-reared males. For males, the effects of physical enrichment on activity were not significant. For females,

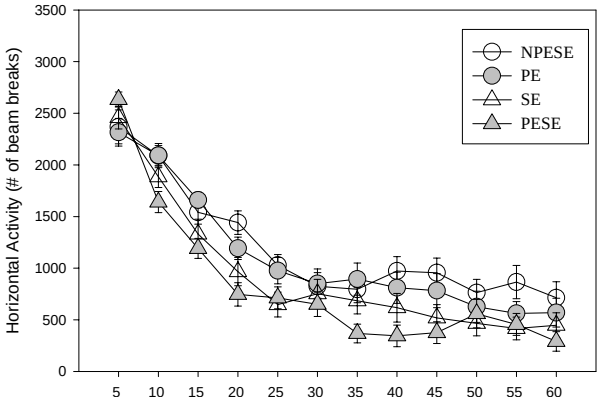

Figure 18. Intact Males: Within session activity ED 17

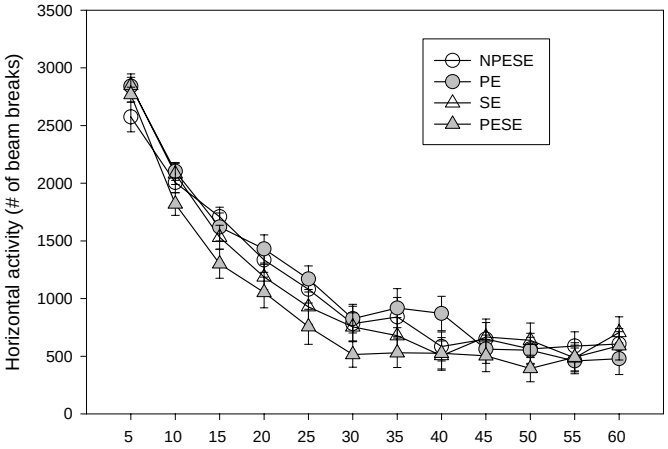

Figure 19. Intact Females: Within session activity ED 17

there were no effects of social or physical enrichment on activity patterns or habituation on ED 17.

Enrichment Day 28. See Figures 20-21. On enrichment day 28, when all animals were analyzed together, there was a significant effect of time with all groups decreasing activity across the testing session [Time: F (11, 1980) = 275.33, p <0.001]. Further, females were more active than were males throughout most of the testing period [Gender: F (1, 180) = 18.99, p < 0.001]. Socially-enriched animals habituated more quickly when compared to isolated animals [Social: F (1, 180) = 8.93, p = 0.05]. When data were analyzed separately within gender, males and females habituated over time to the testing chamber [M-Time: F (11, 968) = 165.71, p <0.001; F-Time: F (11, 1012) = 114.99, p <0.001]. Socially-enriched females [Social: F (1, 92) = 5.65, p =0.05] exhibited less activity over time (greater habituation) when compared to isolated females. The effects of social enrichment on male activity, however, existed only as a trend [Social: F (1, 88) = 3.43, p =0.067]. There were no separate effects of physical enrichment on activity habituation in either males or females on ED 28.

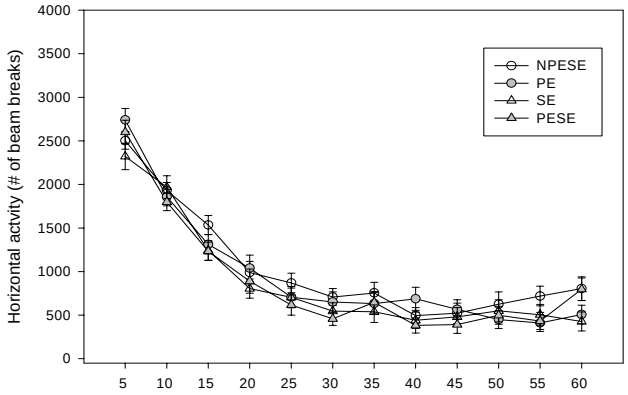

Figure 20. Intact Males: Within session activity ED 28

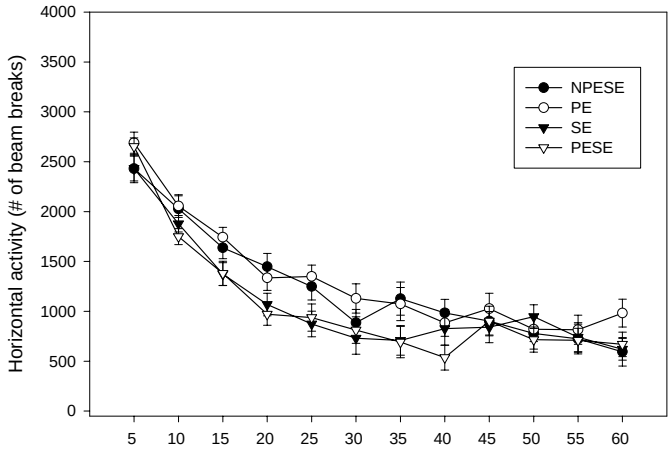

Figure 21. Intact Females. Within session activity ED 28

Locomotion Summary. The effects of social vs. physical enrichment on locomotor habituation depended on time in enrichment (*i.e.,* day of measurement) and animal Sex. On enrichment Day 12 (1st locomotor experience during enrichment), both social and physical enrichment reduced activity in males. Among males, the effect size (eta squared) for social enrichment (17.5%) was greater than the effect size for physical enrichment (5.6%), suggesting that, while physical enrichment does act to improve performance for males, social enrichment may be the most important factor affecting overall performance. Among females, only social enrichment reduced activity, suggesting that for females, social enrichment is the key factor affecting performance and that females may be insensitive to the effects of physical enrichment. Notably, the effect size (eta squared) for social enrichment on ED 12 also was greater in males (17.5%) than in females (9.5%), suggesting that males may be more sensitive than females to enrichment effects on performance in general.

On enrichment Day 17 (2nd locomotor experience), social but not physical enrichment reduced activity and only among males, accounting for 14.6% of activity variance. The effects of physical enrichment disappeared for males on ED 17. These data suggest that males are more sensitive than are females to the early phases of enrichment and that the social aspects of enrichment may be more important than the physical aspects of enrichment for both males and females.

On enrichment Day 28 (3rd locomotor experience), the pattern of activity reversed for males and females. Specifically, social enrichment reduced activity only as a trend in males, accounting for 3.4% of activity variance. In contrast, the

effects of social enrichment to reduce activity returned for intact females, accounting for 5.8% of activity variance. These findings suggest that females may take longer to derive consistent benefit from the effects of enrichment and that social enrichment is responsible for any observed effects.

Overall, these results suggest that social environment is particularly important and that gender differences in enrichment effects exist. Males exhibit an early response to the physical environment, but they are most responsive to social aspects of the environment to which they respond more consistently and robustly. Females, in contrast, appear insensitive to the effects of the physical environment. The next question is do males and females also differ in their responses when assessed on more complex cognitive measures? That is, are the observed gender differences in the effects of social and physical enrichment specific to only certain domains of cognitive functioning or does social enrichment affect both simple and complex cognitive functions?

Acoustic startle reflex (ASR) with and without pre-pulse inhibition (PPI).

The acoustic startle reflex is an index of reactivity to a sudden unexpected acoustic stimulus. The reflex is altered in response to varied emotional states (*e.g.*, stress) and in various neurologically-based disorders (*i.e.*, schizophrenia), including brain injury (Wiley *et al*., 1996; Lu *et al*., 2003). The startle reflex is reduced if it is preceded by a non-startling tone. The preceding tone, although present at a semi-conscious level, is believed to serve as a warning signal, reducing the amplitude or strength of the subsequent reflex. The process by which this preceding tone inhibits

subsequent startle is believed to reflect pre-conscious information processing, sensory gating, or attention.

Analytic approach. ASR and percent prepulse inhibition (% PPI) data (*i.e.,* startle to 120 dB stimuli and the percentage of pre-pulse inhibition to the stimulus when paired with a 75 dB, 82 dB, or visual prepulse) were first analyzed using repeated-measures analyses of variance to evaluate possible changes in startle or % prepulse inhibition over the enrichment period. Percent pre-pulse inhibition was calculated as [(amplitude of trial without pre-pulse) - (amplitude of trial with pre-pulse)/amplitude of trial without pre-pulse] x 100. These calculations are standard (Faraday & Grunberg, 2000; Swerdlow, Braff, & Geyer, 2001; Acri, 1994). Greater startle amplitudes reflect greater reactivity and greater prepulse inhibition reflects greater information processing/sensory gating. Because between-group differences were present when data were analyzed at this level, univariate analyses were conducted on each day.

Baseline analyses. A multivariate analysis of variance (MANOVA) was performed on baseline startle and % PPI (75 dB, 82 dB, and visual) values. Males and females differed significantly in response to the startle stimulus. Therefore, analyses of startle response were run separately for males and females. Additionally, there were some differences in % PPI among the animals assigned to the different enrichment groups. Therefore, subsequent analyses on each of the PPI responses during the enrichment period were run using baseline values as covariates. Also, because of the significant gender differences in startle response, subsequent analyses of % PPI were run separately for males and females.

Startle amplitude from baseline to ED 30. *See Figures 22-23.*

Repeated-measures ANOVAs were used to evaluate changes in performance from baseline to ED 30 on startle and PPI values. Data were analyzed separately within males and females because of gender differences in startle amplitude. Males exhibited increasing startle amplitude over time, suggesting that they became sensitized to the testing environment [Time: $F(3, 276) = 124.41$, $p < 0.001$]. Social and Physical enrichment had no effect on startle amplitude in males. Females exhibited a more variable startle response pattern across time [Time: $F(3, 276) = 30.11$, $p < 0.001$], increasing startle amplitude from baseline to ED 15 and decreasing startle amplitude form ED 15 to ED 20. There were no further changes in startle amplitude after ED 20. There were no main effects of physical or social enrichment on startle amplitude in females. Because there were no significant group differences, univariate analyses were not performed on startle amplitude.

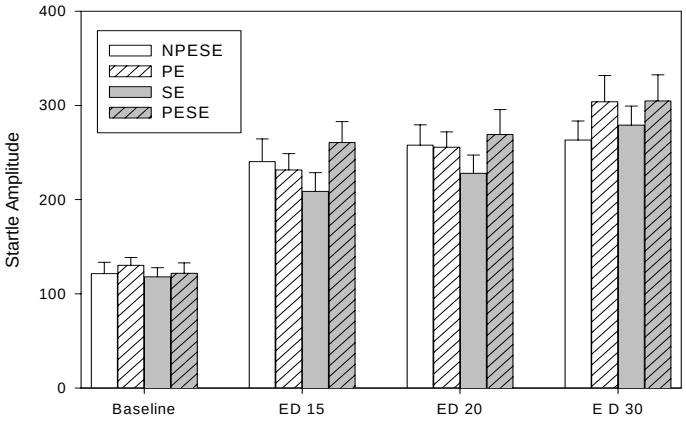

Figure 22. Intact Males: Startle amplitude BL to ED-30

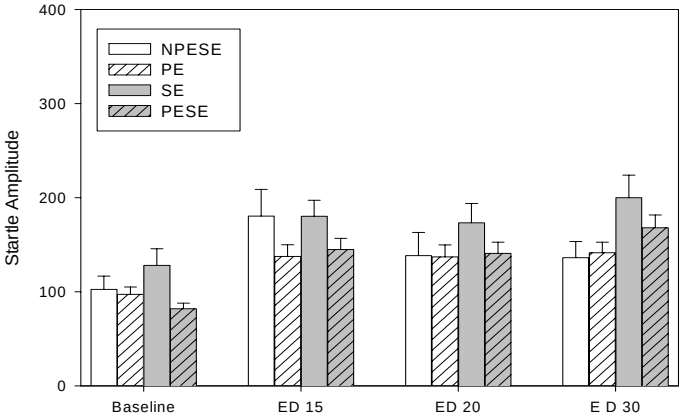

Figure 23. Intact Females: Startle amplitude BL to ED-30

% PPI- 82 dB across the enrichment period. *See Figures 24-25*. Repeated-measures ANOVAS were used to evaluate changes in % PPI across time. Changes in % PPI at the 82 dB level were analyzed first. Again, data were analyzed separately for males and female because of gender differences in startle amplitude. Further, there was a trend for baseline %PPI-82 dB to differ among groups within females; therefore baseline values were used as a covariate when analyses were run within both males and females. Within males, % PPI-82 dB did not change significantly over time. The effects of physical enrichment appeared to depend on the social context, reducing % PPI-82 dB in the isolated environment and increasing % PPI in the social environment [Social X Physical: F (1, 91) = 4.94, p <0.05]. Within females, %PPI-82 dB increased over time [Time: F (2, 182) = 6.55, p <0.05], however, these effects depended on physical enrichment with physically-enriched animals exhibiting a consistent increase in % PPI over time and non-physically enriched animals exhibiting a more variable response pattern over time [Time X Physical: F (2, 182) =

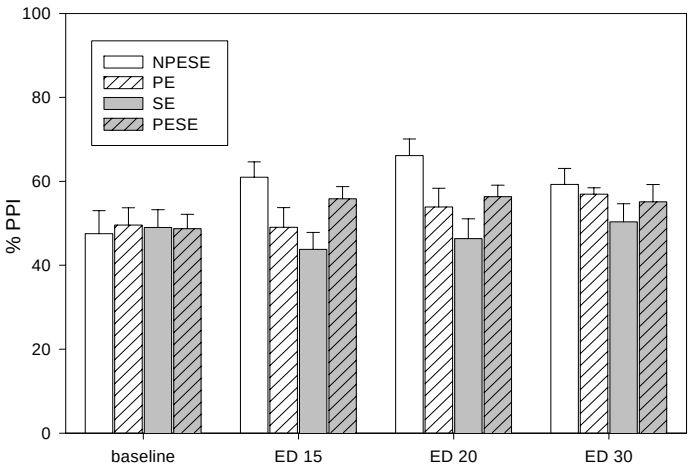

Figure 24. Intact Males: % PPI-82 across days

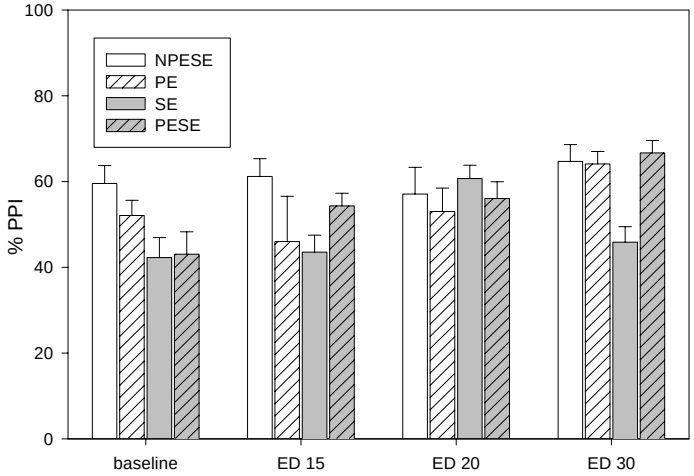

Figure 25. Intact Females: % PPI-82 across days

3.07, p < 0.05]. The main effects of physical and social enrichment in females followed the same pattern as in males with physical enrichment decreasing %PPI-82 dB in the isolated environment, but increasing %PPI-82 dB in the social environment [Social X Physical: F (1, 91) = 4.47, p < 0.05].

% PPI-75 over time. *See Figure 26 -27.* Changes in % PPI at the 75 dB level were analyzed next. As with startle and %PPI-82 dB, data were analyzed separately for males and female because of gender differences in startle amplitude.

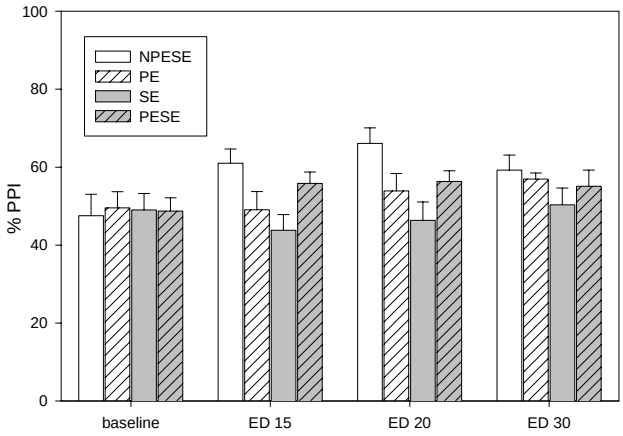

Figure 26. Intact Males: % PPI-82 across time

Because, there were baseline differences among groups on measures of %PPI-75 dB, baseline values were used as a covariate when analyses were run within males and females. Within males, % PPI-75 dB did not change significantly over time. Social enrichment decreased % PPI-75 dB [Social: F (1, 91) = 5.19, p <0.05]. The effects of physical enrichment on % PPI –75 dB appeared to depend on the social context, reducing % PPI-75 dB in the isolated environment and increasing % PPI in the social environment [Social X

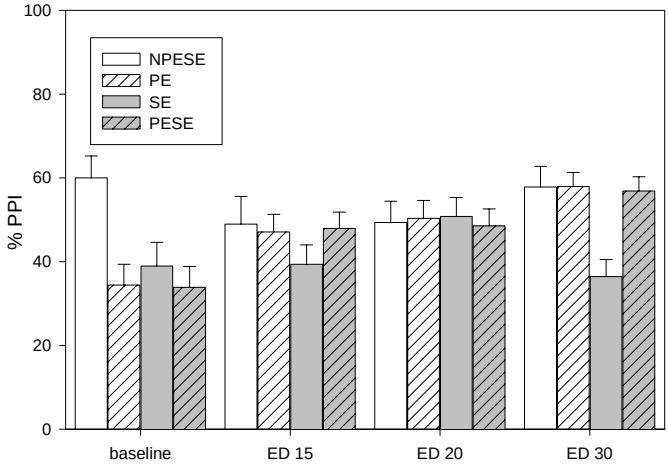

Figure 27. Intact Females: % PPI-82 across time

Physical: F (1, 91) = 10.38, p<0.05]. Within females, %PPI-75 dB increased over time [Time: F (2, 182) = 4.63 p <0.05], however, these effects depended on social enrichment with isolated animals exhibiting a consistent increase in % PPI over time and socially-enriched animals exhibiting a more variable response pattern over time [Time X Social: F (2, 182) = 3.07, p <0.05]. For females, physically-enriched animals had greater %PPI than did non-physically enriched animals [Physical: F (1, 91) = 5.47, p <0.05].

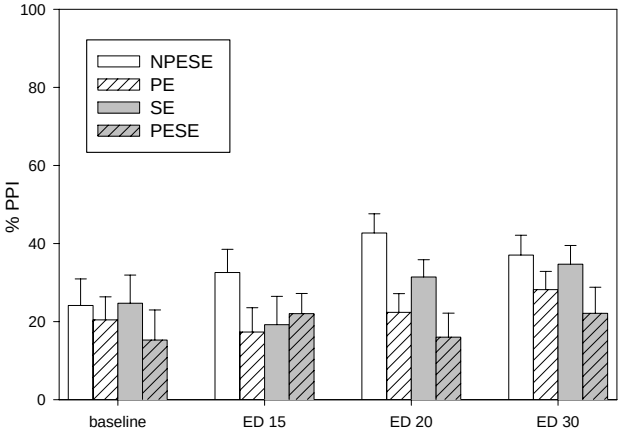

Figure 28. Intact Males: % PPI-visual Across ED 15-30

% PPI-visual over time. *See Figures 28-29.* Within males, there was a trend for % PPI-visual to increase over time [Time: F (2, 182) = 2.402, p =0.093]. Physically-enriched animals had lower % PPI-visual than did non-physically-enriched animals [Physical: F (1, 91) = 6.31, p <0.05]. Within females, %PPI-visual improved over time [Time: F (2, 182) = 8.29, p <0.001]. As with acoustic % PPI, there was a trend for the effects of physical enrichment to depend on the social context with physical enrichment decreasing % visual PPI in the isolated environment and increasing % visual PPI in

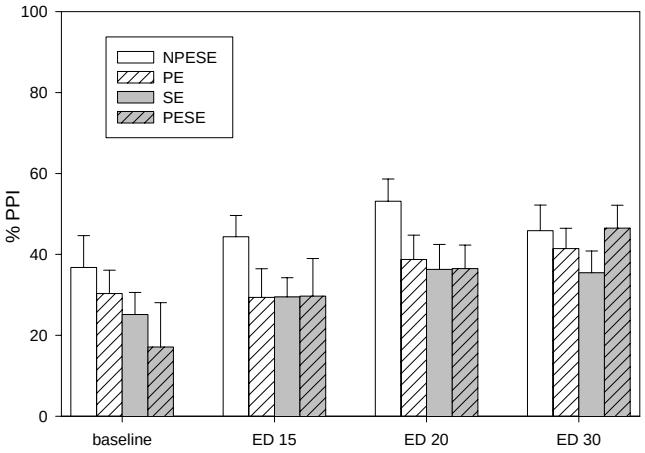

Figure 29. Intact Females: % PPI-visual Across ED 15-30

the social environment [Social X Physical: F (1, 91) = 3.53, p = 0.063]. There was no main effect for social enrichment on % visual PPI in either males or females.

Enrichment Phase Analyses. % PPI 75 dB, % PPI 82 dB, and % PPI visual. Next, because the effects of social and physical enrichment varied across the different PPI modalities, separate univariate analyses were used to examine the effects of social and physical enrichment for each modality of PPI on each enrichment day. Because there was an overall main effect for gender when analyses were performed across time and because the study was designed to examine whether males and females differ in their response to social and physical aspects of the environment, the effects of social and physical enrichment were examined separately within males and females on each measurement day (ED 15, 20, & 30). Results of these analyses are presented for each enrichment day.

Enrichment Day 15: % PPI-82 dB; % PPI-75 dB, and % PPI-visual. As with the previous analyses, males and females were considered separately and univariate analyses were run using baseline values as covariates. For males, on enrichment Day 15, there was a significant Social X Physical interaction on acoustic pre-pulse levels 82 dB [Social X Physical: F (1, 91) = 6.92, p <0.05] and 75 dB [Social X Physical: F (1, 91) = 10.58, p <0.05] similar to that reported in previous analyses. For males, socially-enriched animals had lower % PPI (82 dB) than did isolated animals [Social: F (1, 91) = 4.65, p <0.05]. Within females the Social X Physical interaction existed as a trend to affect % PPI-82 dB only [F (1, 91) = 3.49, p =0.065]. There also was a trend for physically-enriched animals to have greater %

PPI-75 dB than non-physically enriched animals [Physical: F (1, 91) = 3.33, p = 0.071]. Social and Physical enrichment had no effect on % visual PPI.

Enrichment Day 20: % PPI-82 dB; % PPI-75 dB, and % PPI-visual. Data analyses followed previous patterns. Within males, socially-reared animals exhibited less % PPI-75 dB [F (1, 91) = 5.07, p <0.05] than did isolated animals. This same effect existed as a trend for visual pre-pulse [F (1, 91) = 2.93, p =0.090]. Physical enrichment also significantly reduced % visual PPI [F (1, 91) = 11.21, p <0.05]. These effects were present in both the isolated and social context. In contrast, the effect of physical enrichment on the acoustic % PPI parameters differed depending on context with physical enrichment decreasing % PPI in the isolated environment only [% PPI-82 dB: F (1, 91) = 5.03, p <0.05; % PPI-75 dB: F (1, 91) = 8.73, p <0.05]. Within females there were no effects of social or physical on startle or % PPI values.

Enrichment Day 30: 82 dB PPI, 75 dB PPI, and Visual PPI. When males and females were considered separately, within males, there was no effect of social or physical enrichment on % PPI (acoustic or visual). Within females socially-enriched animals exhibited less inhibition to acoustic pre-pulse [% PPI -75: F (1, 91) = 5.27, p <0.05]. In contrast, physical enrichment increased % PPI at both the 82db [F (1, 91) = 11.16, p =0.001] and 75 dB [F (1, 91) = 10.69, p = <0.05] levels. These effects followed a similar pattern from previous days in that the effects of physical enrichment to enhance % PPI were present in the social environment only [% PP-82 Social X Physical: F (1, 91) = 9.15, p <0.05; % PP-75 Social X Physical: F (1, 91) =

4.26, p = <0.05]. There were no effects of physical or social enrichment on % visual PPI.

ASR and PPI summary

Overall, as with locomotor activity, the effects of enrichment on ASR and % PPI varied in relation to the length of time in enrichment and animal gender. Males, again, were more sensitive to the early effects of social enrichment with isolated animals catching up by the last measurement day (enrichment day 30). Social enrichment consistently reduced % PPI for males. Further, for males, the effects of social enrichment to reduce PPI were somewhat buffered by the presence of physical objects with animals reared in a combined social and physical environment (PESE) exhibiting greater PPI than animals reared in the social-only environment (SE). In females, a similar pattern was present with animals reared in the complex social environment exhibiting greater % PPI than animals in the SE environment, but less % PPI than animals reared in an isolated environment.

On the second ASR exposure, enrichment day 20, the effects of social enrichment to reduce acoustic % PPI remained in males and a new effect of physical enrichment to reduce % visual PPI appeared. The effects of physical enrichment on % visual PPI was present in the social and isolated environments, whereas the effect of physical enrichment on acoustic % PPI depended on the social context. There were no effects of enrichment on % PPI (acoustic or visual) in females on enrichment day 20. On the final ASR test day (enrichment day 30), the effects of physical to reduce visual PPI remained as a trend only in males. In contrast, the effects of social and physical enrichment to affect PPI reemerged in females. Social

enrichment reduced % PPI at both the 75 dB and 82 dB levels. A new effect for physical enrichment to increase acoustic PPI appeared, but again these effects depended largely on the social context, buffering the low % PPI levels in the SE group.

Overall, males appeared more sensitive to the enrichment effects, showing earlier, more robust results that persisted until enrichment day 20 and then disappeared. Females exhibited little response to enrichment early on, but effects appeared and were robust on enrichment day 30. In contrast to males, in which social enrichment consistently decreased % PPI over time, for females, physical enrichment increased % PPI over time, and significantly increased PPI by enrichment day 30. There was no significant effect of enrichment on startle amplitude during the enrichment period. These findings may suggest that for males, social enrichment has no beneficial effect on sensory gating, but for females, physical enrichment acts to improve sensory gating.

Passive Avoidance Performance: Intact Females

After all other dependent measures were gathered, 48 neurologically-intact females assigned to N-PESE, PE, SE, or PESE housing conditions were tested in a passive avoidance task to determine whether this additional measure of simple memory would be useful in future experiments. Passive avoidance is a simple working memory task consisting of a training day and a testing day. On the training day, each animal is placed into one chamber of the shuttlebox. After an acclimation period, a light goes on, and the door opposite the still-dark chamber opens. When

animals cross into the dark component, a mild footshock (0.40 mA) is delivered through the grid floor. Twenty-four hours later, animals are tested using the same procedure; however, no shock is delivered if animals cross into the darkened chamber. Latencies to cross into the dark chamber on the testing day are interpreted as behavioral evidence of memory (*i.e.,* the animals remembers the shock from the previous day). Longer latencies to cross or not crossing into the chamber at all on the second (testing) day indicate better memory function.

Passive avoidance was chosen in this experiment to provide an intermediate measure of cognitive performance with a level of complexity between non-conscious ASR and PPI responses, simple information processing measured by locomotor habituation, and spatial memory as measured by the Morris water maze.

Analytic Approach

Training latencies were compared with testing latencies using Wilcoxon Signed Rank Tests (nonparametric t-tests) because latencies did not meet parametric test criteria (*i.e.*, homogeneity of variance, normal distribution). Because latency data were bounded (a maximum value of 300 seconds) and did not meet criteria for parametric tests, training and testing latencies were analyzed with Kruskal-Wallis nonparametric tests. Then, because maximal memory for the aversive event is indicated by the animal not crossing into the darkened chamber at all, testing latencies also were recoded into a binary format in which each animal's performance was scored as "crossed" or " did not cross." These data were analyzed with chi-squares to determine whether the proportion of animals that did not cross was significantly greater than chance for specific groups and subgroups.

Task-validity. *See Figure 30.*

Before pursuing between-subject analyses, training latencies were compared with testing latencies to validate that learning had occurred. That is, did animals demonstrate memory for the aversive event that had occurred 24 hours earlier in the dark chamber by taking longer to cross into the dark chamber on the testing day? Testing latencies were significantly longer than training latencies when all subjects were considered together ($Z = -4.99$, $df = 48$, $p < 0.05$) as well as for subgroups indicating that memory had occurred. For NPESE subjects, the difference between training latency and testing latency existed only as a trend ($Z = -1.88$, $df = 12$, $p < 0.05$), suggesting that this group exhibited less learning than did the other three groups.

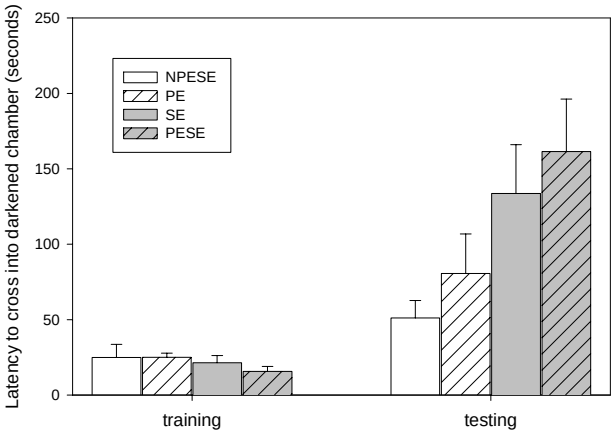

Figure 30: Intact Females: Passive avoidance training and testing latencies

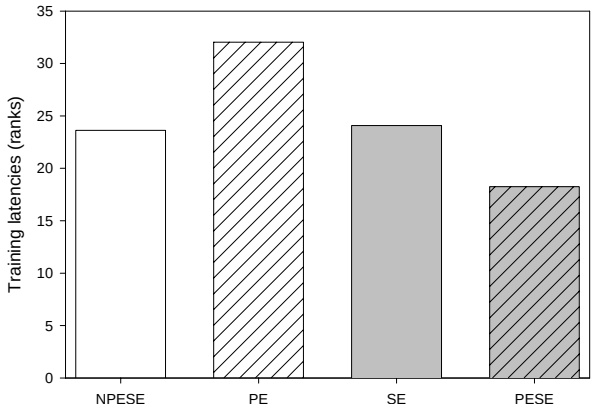

Figure 31: Intact Females: Mean rank scores of passive avoidance training latencies

Training latencies. *See Figure 31.*

When all animals were considered together, the four subgroups did not differ in their latencies to cross into the darkened chamber.

Testing latencies. *See Figure 32*. As with training latencies, test latencies were examined using non parametric ANOVAs to examine the effects of physical and social enrichment to affect latency to cross into the darkened chamber. Latency on the testing day is interpreted as evidence of memory. Animals that take longer to cross or do not cross into the darkened chamber are said to have better memories than animals that cross quickly to the other side. For intact females, animals reared in social environments had significantly greater latencies to cross into the darkened chamber on the testing day than did animals reared in isolation [Z = 5.74, df =1, p <0.005]. Physical enrichment did not significantly affect performance on this measure.

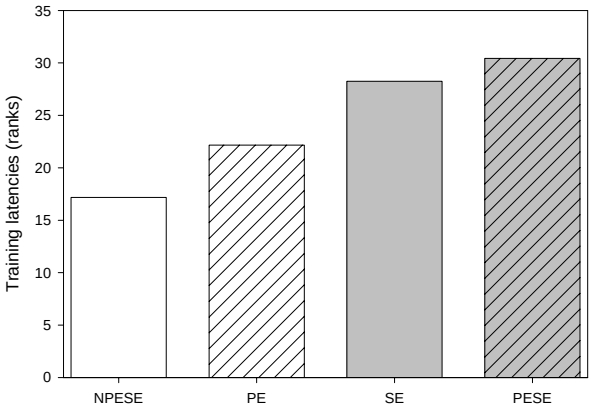

Figure 32: Intact Females: Mean rank scores of passive avoidance testing latencies

Chi-Square Analyses. *See Table 3*. Because maximal memory for the task is indicated by the animal not crossing into the darkened chamber at all, comparisons were made between proportions of animals not crossing (*i.e.,* animals that remembered). When all animals were considered together, more animals did not remember (*i.e.,* crossed) than remembered (*i.e.,* did not cross). Comparisons within specific subgroups indicated that significantly more animals crossed than did not cross in each subgroup except the PESE (*i.e.,* combined social and physical) group. Together, these findings suggest that the PESE group may have had the greatest effect to improve memory.

Table 3. Experiment I: Number of animals that did not remember (i.e., crossed into the dark chamber) vs. remembered (i.e., did not cross) on the testing day.

Group Tested	**# crossed**	**# did not cross**	**Chi-square(df)**	p value
All Animals	41	7	24.083 (1, 48)	p <0.001
Female-NPESE	12	0	*	
Female-PE	11	1	8.333 (1, 12)	p <0.05
Female-SE	10	2	5.333 (1, 12)	p <0.05
Female-PESE	8	4	1.333 (1, 12)	p = .240

* a chi square could not be calculated because all animals crossed

Passive Avoidance Summary. Overall, similar to the locomotor activity and ASR/PPI data, social enrichment had the greatest effect to alter performance with socially-reared animals performing better than isolated animals on this simple memory task. Physical enrichment did not improve performance of intact females.

Morris Water Maze: Intact Animals

The Morris water maze is a complex cognitive task that was used in this experiment (enrichment days 22-26) to index spatial learning and spatial memory. The water maze task procedure used in this experiment was based on procedures previously used in the enrichment literature. The task was conducted across 5 days with 4 trials on each day. Three minutes separated each trial. The platform remained in the same position across all five days, but the release point of the animals varied across each trial on each day. Animals could not just memorize the swim path. They had to learn the position of the platform. Because the platform remained in the same location for the duration of the experiment, trial 1 of each day after the first testing day was used to index long-term memory.

Analytic Approach. Learning was assumed to have occurred if, over several trials, animals swam more quickly and directly to the visible platform. The platform remained in the same position for each trial, but the release position of the animal was randomly varied. Paired t-tests comparing average trial 1 latency and distance to trial 4 latency and distance were used to establish task validity. That is, performance on the last trial of each day should be better than the first trial of each day if animals are learning where the platform is located.

Further evidence of learning is provided by examining latencies to the platform on the first trial of each day after the first test day (test days 2–day 5). Latency to find the platform on the first trial of day one was not included in these analyses, because it was the first water maze exposure for all animals.

Shorter latencies to find the platform on the first trial of each day are evidence that the animals have retained the position of the platform in long-term memory store (between days; 24-hours). Distance to find the platform on trial 1 also should decrease over time as the animal learns that the position of the platform does not change and therefore takes a more direct route to locate the platform. Repeated analyses of variance were used to examine trial 1 performance across the four days of testing (test days 2-5) and to determine if changes over time differed depending on animal group. If there were between-group effects, then univariate analyses were performed on each day.

Finally, to examine whether overall performance increased over the 5 days, repeated measures analyses were used to examine changes in mean latency to find the platform and mean distance traveled to the platform across test days 1-5 (ED 22-26). To parallel the previous data analytic strategies, group differences on mean performance (latency and distance to platform) were examined on each day.

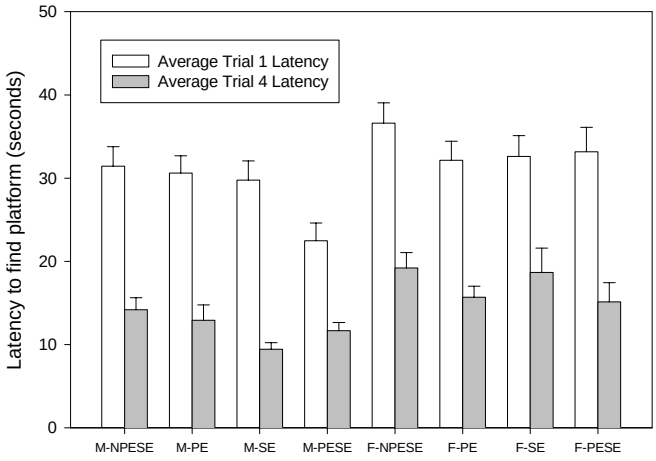

Figure 33. Intact Animals: Latency to find platform on Trial 1 and Trial 4 averaged over ED 22-26

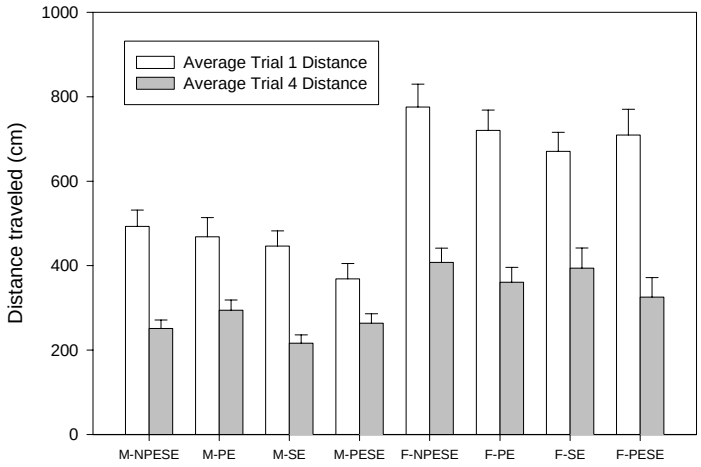

Figure 34. Intact Animals: Distance to find platform on Trial 1 and Trial 4 averaged over ED 22-26

Task Validation. *See Figures 33-34.*

When all animals were analyzed together, paired t-tests indicated that performance improved from trial 1 to trial 4 for all animals (latency: $t = 18.03$, $df = 191$, $p < 0.001$; distance: $t = 14.04$, $df = 191$, $p < 0.001$). These effects remained when analyses were split by gender and group.

***Morris Water Maze: Performance across Time*.** *See Figures 35-36*. Repeated measures ANOVAs were used to examine changes in mean latency and distance to find the platform across the five testing days (ED 22-ED 26). When all animals were analyzed together, there was a main effect for Time such that mean latency [F (4, 736) =160.80, p<0.001] and the distance traveled [F (4, 736) =95.12, p <0.001] to find the platform improved from Day 1 to Day 5 (ED 22 – ED 26), suggesting that the animals were finding the platform faster and learning a more direct route to the platform over time. Males had shorter latencies [Gender: F (1, 184) = 20.13, p <0.001; Gender X Time: F (4, 736) = 6.54, p <0.001] and swam shorter distances [F (1, 184) = 81.68, p <0.001] than did females. There also was a trend for physically enriched animals to have shorter latencies to find the platform than non- physically-enriched animals [Physical: F (1, 184) = 3.41,

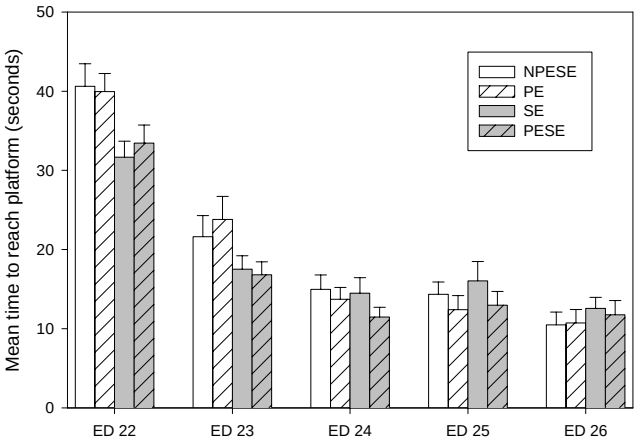

Figure 35. Intact males: Mean latency to reach platform on ED 22-26

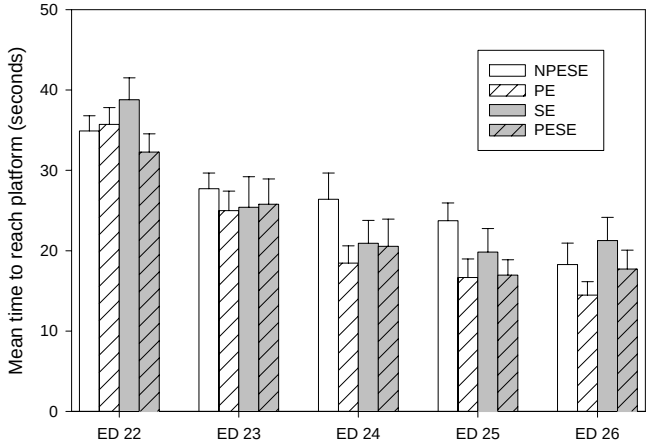

Figure 36. Intact females: Mean latency to reach platform on ED 22-26

p =0.066]. When collapsed across time, there was no overall main effect of social enrichment on latency or distance traveled. However, the effects of social enrichment on mean latency to find the platform did vary over time at this level of analysis [Time x Social: F (4, 736) = 3.31, p <0.05]. Because there was a main effect for gender, the effects of social and physical enrichment also were evaluated separately within males and females. Within males, the effects of social enrichment to improve latency to find the platform existed as a trend [F (1, 92) = 3.51, p =0.064]. In contrast, for females, physical but not social enrichment [F (1, 92) = 3.44, p =0.067] improved latency to find the platform over time.

Next, because the effects of social varied across time and because there was an overall trend for physical enrichment to improve performance, the separate effects of social and physical enrichment were examined on each day. Further, because males were generally faster and traveled less distances than females, the effects of social and physical were separately examined within males and females on each measurement day.

WM Day 1 (ED 22).

Spatial memory and learning were evaluated by examining the mean latency to find the platform on each day. Shorter latencies and shorter distances to find the platform indicate that the animal has learned the position of the platform. Within males animals reared in socially-enriched environments (PESE and SE) had shorter latencies to find the platform than animals in non-social environments [F (1, 92) =10.61, p< 0.05). There was no effect of social or physical enrichment on latency to reach the platform in females.

WM Day 2 (ED 23).

Within males, animals reared in socially-enriched environments (PESE and SE) had shorter latencies to find the platform than animals in non-social environments [$F(1, 92) = 5.80$, $p < 0.05$). Within males, there was also a trend for animals reared in the social environment to travel shorter distances to reach the platform than animals reared in non-social environments [$F(1, 92) = 3.21$, $p = 0.077$]. There was no effect of social or physical enrichment alone or in combination on latency to reach the platform or distance traveled to reach the platform in females.

WM Day 3 (ED 24).

There were no effects of physical or social enrichment on latency to reach platform or distance traveled to reach the platform within either males or females.

WM Day 4 (ED 25).

Within males, there were no effects of social or physical enrichment on latency or distance traveled to find platform on Day 4. In contrast, a significant effect for physical enrichment to reduce latency to find platform appeared for females [$F(1, 92) = 4.44$. $p < 0.05$].

WD Day 5 (ED 26).

There were no effects of physical or social enrichment on latency to reach platform or distance traveled to reach the platform within either males or females.

Performance on Trial 1 as a Measure of Long-Term Memory. *See Figures 37-38.*

Because the platform remained in the same position on each day, shorter latencies and shorter distances to find the platform on trial 1 on each day after the first testing day suggests the animals have retained the position of the platform in memory in long-term memory store. When all animals were analyzed together, latencies to find the platform [Time: F (4, 736) = 94.22, p <0.001] and distance traveled to the platform [Time: F (4, 732) = 32.44, p <0.001] improved across testing days 1-5. Males had shorter latencies than females to find the platform on trial 1 collapsed across days [F (1, 184) = 8.93, p<0.001] and shorter distances to find the platform [F (1, 183) = 68.73, p <0.001]. There also was a trend for social enrichment to reduce the latencies [F (1, 184) = 3.57, p =0.061] and distance traveled [F (1, 183) = 3.76), p =0.054] to find the platform on trial 1 collapsed across days. There was a similar trend for physical enrichment [F (1, 184) = 3.14, p =. 078] to reduce latency to find the platform. Because there was a main effect for gender, the effects of physical and social

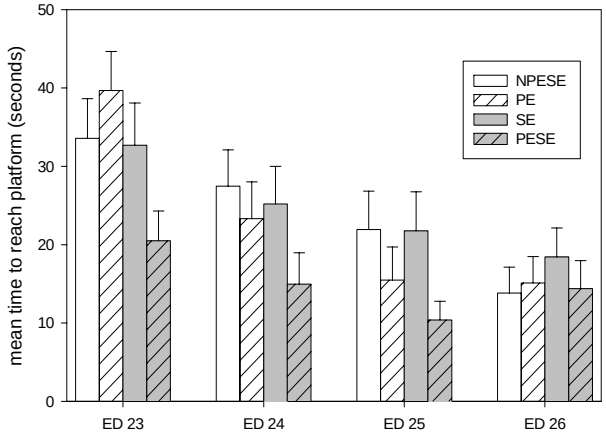

Figure 37. Intact Males: Mean time to reach platform on Trial 1, ED 23-26

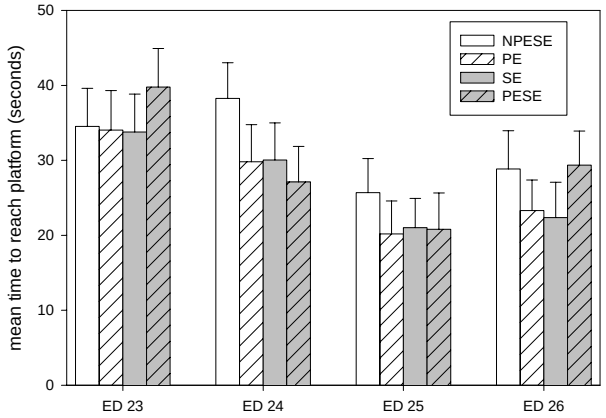

Figure 38. Intact Females: Mean time to reach platform on Trial 1, ED 23-26

enrichment were analyzed further within males and females. Within males, performance on trial 1 improved across successive test days (Time) [Latency Time: $F(4, 368) = 67.76$, $p < 0.001$; Distance Time: $F(4, 368) = 14.981$]. Socially-reared animals had shorter latencies to find the platform [Social: $F(4, 368) = 4.88$, $p = 0.030$] than isolated animals. There also was a trend for animals reared in a physically-enriched environment to have shorter latencies than animals reared in non-physically-enriched environments [Physical: $F(4, 438) = 3.34$, $p = 0.071$]. Within females, latency to find the platform and distance traveled to find the platform on trial 1 improved across testing days [Latency Time: $F(4, 368) = 32.09$, $p = <0.001$; Distance Time: $F(4, 368) = 19.40$, $p = <0.001$]. There were no main effects of social or physical enrichment on latency to find the platform or distance traveled to find the platform in females.

Next, because distances and latencies to reach platform varied considerably within groups, latencies to find platform also were analyzed in a binary format with data coded based on a median split of mean performance times averaged across days (*See Table 4*). Animals who found the platform in less than the median time were considered “good performers,” whereas animals that found the platform in greater than the median time were considered “poor performers.” When all animals were considered together, males were better at the task (# “good” performers > # “poor” performers) than were females (# “poor” performers > # “good” performers). Comparisons within specific subgroups indicated that for males social enrichment appeared to have the greatest impact to improve performance. Specifically, for males, animals in the SE and PESE groups had a significantly greater number of

"good" performers than "poor" performers. Performances did not differ in isolated groups. For females, the pattern was slightly different in that the isolated environment appeared to have deleterious effects on performance with a greater number of "poor" performers than good performers in the NPESE group. The social and physical environment appeared to buffer these effects in that within the PE, SE and PESE groups there was no significant differences between the number of "good" performers and the number of "poor" performers.

Table 4. Number of good performers (*i.e.*, <19.93seconds to find platform) vs. poor performers (> 19.93 seconds to find platform) based on grand mean across days.

Group Tested	**"Good" Performers**	**"Poor" Performers**	**Chi-square(df)**	**p value**
All Males	61	35	7.042 (1)	p <0.001
Male-NPESE	11	12	0.167 (1)	p = 0.683
Male-PE	14	10	0.667 (1)	p =0.414
Male-Se	18	6	6.000 (1)	p <0.05
Male-PESE	18	6	6.000 (1)	p <0.05
All Females	35	61	7.042 (1)	p <0.05
Female-NPESE	6	18	6.000 (1)	p <0.05
Female-PE	8	16	2.667 (1)	p= 0.102
Female-SE	11	13	0.167 (1)	p =0.683
Female-PESE	10	14	0.414 (1)	p=0.414

Morris Water Maze Results: Intact Animals

All groups within both males and females exhibited improved performance over trials 1-4 and over time (testing days 1–5), suggesting that all animals were learning the position of the maze and retaining it in immediate or short-term memory and long term memory. As with the more basic cognitive tasks (information processing and attention), social enrichment appeared to have the greatest influence on this more complex measure of cognitive performance. In addition, the pattern of

effects paralleled effects on the other measures with males responding earlier than females to enrichment. For males, the non-socially enriched animals caught up to the enriched animals after 2-3 exposures to the task, performing similarly to the socially-enriched animals. For females, in contrast, effects of social and physical enrichment were more variable. The results for females were more similar to those observed in ASR in that the physical enrichment appeared to have a greater impact on the performance of females, improving performance over time and with the greatest effects occurring on Day 4.

Overall, the effects of enrichment on learning appeared to depend on length of time in enrichment. Specifically, males exhibited effects of enrichment early, but the non-enriched animals eventually caught up. Females, in contrast, exhibited variable results with effects occurring later and then disappearing (i.e., differences in performance between enriched and non enriched animals disappeared). The effects of enrichment on trial 1 performance followed a similar trend with social and physical enrichment having the greatest effect to reduce latency to find the platform in males, but having no effect in females.

Experiment II: Injured Animals

Summary of Results

In Experiment II, animals underwent brain injury on experimental day 10. Following the injury, animals were placed in one of the four housing conditions (NPESE, PE, SE, or PESE) as previously described. Groups were counterbalanced based on % PPI-82 dB and total horizontal activity at baseline. Animals remained in the different housing conditions for 11 days prior to behavioral testing. During this 11-day period, animals were handled only for the purposes of changing cages. Cages were changed and toys replaced 3-4 x/week. The procedures in Experiment II were the same as in Experiment I with the exception that all animals (males and females) went through the passive avoidance procedures on ED 33-34. Before presentation of the results from Experiment II, data are presented which verify that injury occurred. As with Experiment I, results are presented in the order of increasing task complexity. Data analytic strategies are the same as that used in Experiment I unless otherwise noted.

Verification of Injury

Injury validation was made in two primary ways: 1) neuroscore test (paired t-test comparing neuroscores before and after injury); 2) differences in performance on selected behavioral measures. The neuroscore test is a well-established test designed to validate severity of injury following fluid percussion (Dixon *et al*., 1987). Testing is conducted prior to injury to obtain baseline data and 1-hour following

injury to validate injury. Results from the neuroscore tests have been found to correlate directly to the severity of the damage following the fluid percussion injury (Dixon *et al*., 1987; McIntosh, *et al*., 1987).

The neuroscore (*i.e*., neurobehavioral test) is a composite score of neuromotor function where the maximum score is 20 points. Following the injury, motor abilities are tested and scored by the examiner. Scoring for the animals ranges from 0 (severely impaired) to 4 (normal) on each of the following indices: right and left forelimb flexion, left and right hind limb flexion, resistance of lateral pulsion to the left and right, ability to stand on an inclined plane in angles up to 40^{o} (Dixon *et al*., 1987).

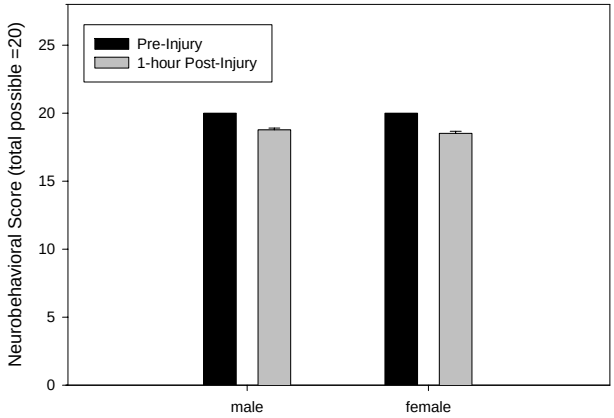

Figure 39. All Injured Animals: Pre and post-injury neuroscores

Neurobehavioral Test. *See Figure 39.*

Paired t-tests were used to compare scores obtained just prior to injury with scores obtained 1-hour following injury. At first, all animals were analyzed together. Then, the effects of injury were examined separately for males and females. When all animals were analyzed together, scores after treatment were lower than scores before treatment ($t = 15.90$, $df = 95$, $p < 0.001$). When animals were analyzed separately by gender, post-injury scores again were lower than pre-injury scores for both males and females (males: $t = 11.28$, $df = 46$, $p < 0.001$; females: $t = 11.27$, $df = 48$, $p < 0.001$). To insure there were no significant differences in the scores among

the groups prior to placement in enrichment, univariate ANOVA was used to compare neuroscores across the treatment groups. There were no group differences in post neuroscores for either males [F (3, 43) = .081, p =0.969] or females [F (3, 48) = 0.88, p = 0.460].

Behavioral Testing

To further corroborate injury effects, the performance of intact animals was compared to the performance of injured animals. Acoustic startle amplitude was evaluated because previous studies have revealed that injured animals have lower startle amplitudes than intact animals (Wiley *et al.,* 1996). Univariate ANOVA was used to compare startle amplitude values between injured and non-injured animals. The Morris water maze was chosen as an additional behavior measure to evaluate injury effects because it is sensitive to the effects of neurological damage (Hooge & De Deyn, 2000). Morris water maze performance also has been used in previous studies and produced reliable effects of injury (Hooge & De Deyn, 2000; Passineau *et al*., 2000).

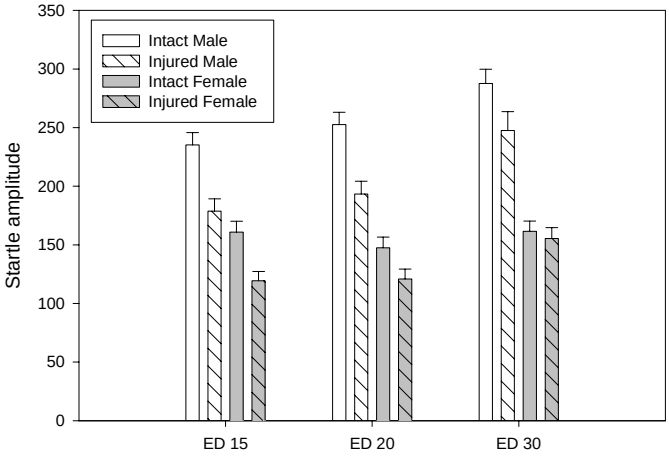

Figure 40. All Animals: Validation of Injury-Startle Amplitude

***Validation of Injury: Startle Amplitude*.** *See Figure 40.*

Before post-injury values were compared, baseline startle amplitudes were compared to ensure that any post-injury differences could not be attributed to differences in baseline startle amplitude levels. When all animals were analyzed

together, there were no differences between the groups (*i.e.,* intact vs. injured) in startle amplitude at baseline. On the first measurement of startle amplitude post injury (*i.e*., 11 days post injury), males startled more than females [Gender: F (1, 283) = 36.54, p <0.001] and injured animals startled less than intact animals [Injury: F (1, 283) = 19.64, p <0.001]. Overall, injury accounted for 6.5 % (eta squared = 0.065) of amplitude variance. Because there was a main effect for gender, the effects of injury were examined separately for males and females. Male [Injury: F (1, 141) = 11.36, p <0.05] and female [Injury: F (1, 141) = 8.25, p <0.05] injured animals had lower startle amplitudes than intact animals. For males, injury accounted for 7.5 % (eta squared = 0.075) of amplitude variance. For females, injury accounted for 5.5% of startle amplitude variance.

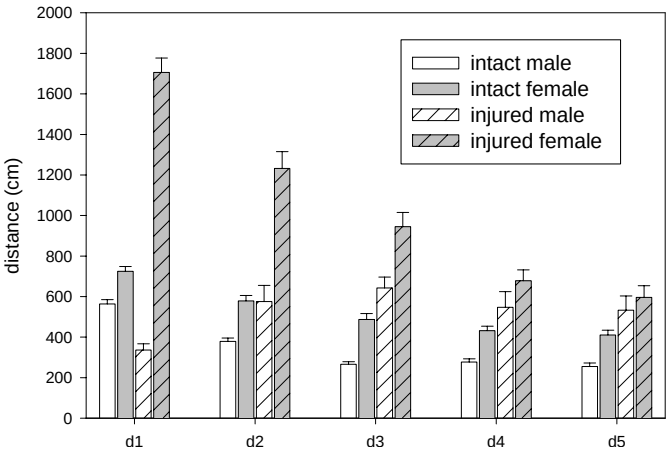

Figure 41. All Animals: Validation of Injury; Distance to find platform ED 22-26

Validation of Injury: MWM Mean Distance Traveled. *See Figure 41.*

When all animals were analyzed together using a multivariate ANOVA (MANOVA), injured animals had longer distances to locate the platform on each water maze day (ED 22-26), suggesting that they had not learned the position of the platform as quickly as intact animals. F values and p values for these analyses are reported in Appendix C. The effects of injury remained when data were examined separately for males and females. In addition to injured animals traveling longer

distances to find the platform, injured animals had longer latencies to find the platform on Trial I. Longer latencies on Trial 1 are suggestive of impairments in the transformation of information into long-term memory store, suggesting further impairments in long-term memory. F values, degrees of freedom, and p values appear in Appendix C.

Experiment II: Injured Animals

Locomotion. *See Figures 42-43*

As with Experiment I, horizontal activity was measured in this experiment as an index of simple information processing. When placed in the activity chambers, activity levels are high. As the animal acclimates to the testing chamber, activity levels drop and eventually level off. Persistent high levels of activity suggest less acclimation and less efficient processing of environmental cues. In experiment II, activity was measured for 1 hour at four separate time points (baseline, ED 12, ED 17, and ED 28).

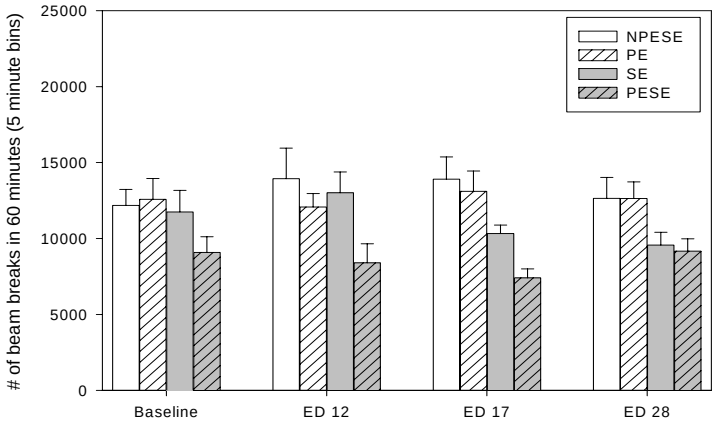

Figure 42. Injured Males: Horizontal activity across ED 12-28

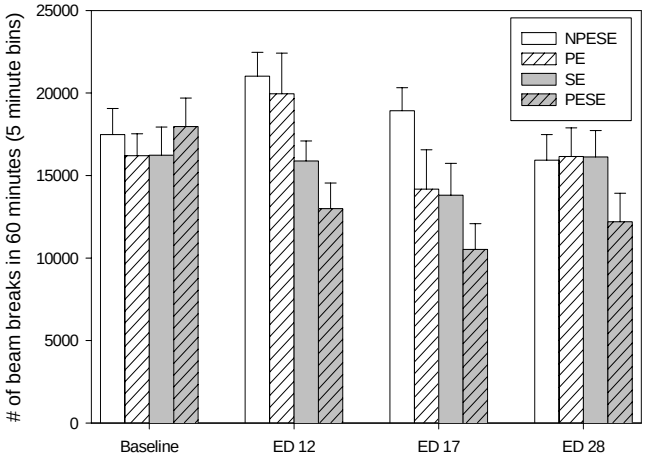

Figure 43. Injured Females: Horizontal activity across ED 12-28

First, all animals were analyzed together using repeated-measures analyses of variance (ANOVA) from baseline to ED 28. When animals were analyzed together, levels of activity varied over time [Time: $F(3, 246) = 5.33$, $p < 0.05$]. Females were more active than males [$F(1, 82) = 28.11$, $p < 0.001$] and isolated

(NPESE and PE) animals were more active than socially-enriched (PESE and SE) animals [Social: $F(1, 82) = 10.33$, $p < 0.05$]. There also was a trend for physical enrichment to reduce activity [Physical: $F(1, 82) = 3.15$, $p = .080$]. Because there was a main effect for gender, the effects of social and physical enrichment were examined separately for males and females. For males, activity levels remained relatively stable across enrichment days. Isolated animals were more active than socially-enriched animals [Social: $F(1, 43) = 11.43$, $p < 0.05$] and there was a trend for physical enrichment to reduce activity [Physical: $F(1, 43) = 3.20$, $p = 0.080$]. For females, activity levels varied across the enrichment period and these effects depended on housing condition [Time: $F(3, 117) = 6.02$, $p < 0.001$; Time X Social: $F(3, 117) = 8.03$, $p < 0.001$; Time X Physical: $F(3, 117) = 3.45$, $p < 0.05$]. Because there were significant between-subject effects in males and Time X Physical and Time X Social interactions in females, univariate analyses were conducted on each day. First, analyses were conducted on baseline activity levels to ensure that activity levels were similar among groups.

***Baseline analyses*.** *See Figure 44*. When all animals were considered together, females were more active than males [Gender: $F(1, 88) = 30.23$, $p < 0.001$]. Activity levels did not significantly differ across groups even when data were analyzed separately for males and females. Therefore, baseline activity levels were not used as a covariate when univariate analyses were run on each day.

*Horizontal Activity: Enrichment Day 12***.** *See Figure 45.* Data from 6 animals out of a total of 98 animals (3 NPESE female; 3 PESE female) were not valid because of equipment or software failure. Another 2 animals died because of complications resulting from surgery or head injury (1 male PE; 1 female SE). Therefore, a total of 8 animals were dropped from the overall analyses. When all animals were considered together, females were more active than males [Gender F (1, 82) = 23.54, p <0.001], socially-enriched animals were less active than non-socially enriched animals [Social: F (1, 82) = 13.03, p <0.001], and physically enriched animals were less active than non-physically enriched animals [Physical: F (1, 82) = 5.10, p <0.05].

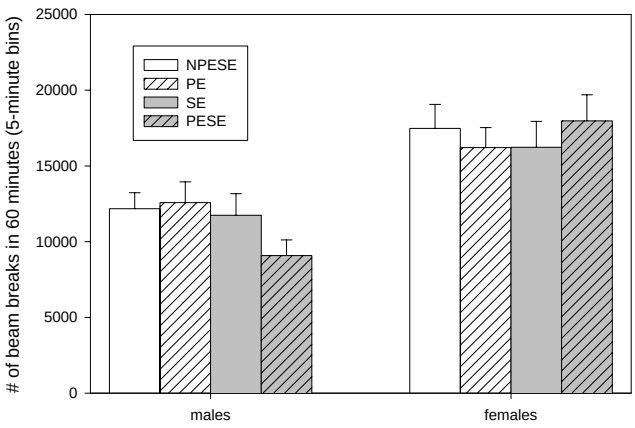

Figure 44. Injured Animals: Baseline Horizontal

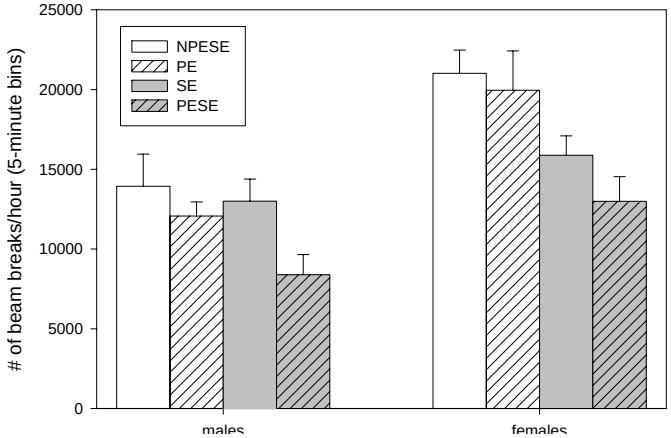

Figure 45. Injured Animals: Horizontal Activity ED 12

When the sexes were considered separately, group differences were present for both males and females. However, the separate effects of social and physical enrichment differed for males and females. Specifically, for males, only physical enrichment had an effect on locomotor activity such that physically-enriched animals were less active than non-physically enriched animals [Physical: F (1, 43) = 4.91, p < 0.05]. In contrast, for females, social but not physical enrichment had an effect on

activity such that isolated animals were more active than socially-enriched animals [Social: F (1, 39) = 11.10, p <0.05]. In contrast to the enrichment effects observed in intact animals, among injured animals, the initial effects of enrichment on activity were more robust in females than in males with social enrichment accounting for 22% of activity variance in females and physical enrichment accounting for only 10% of activity variance in males.

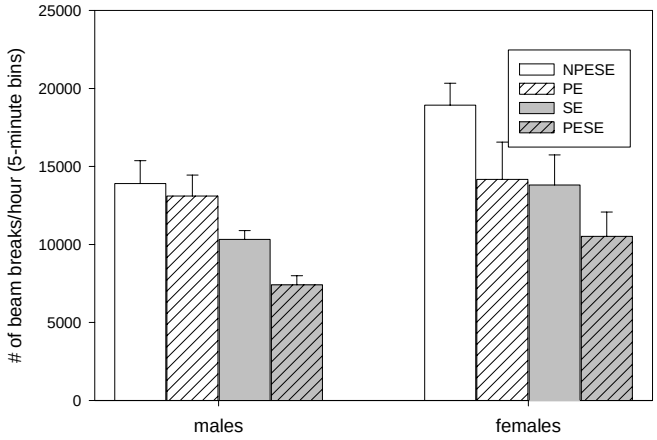

Figure 46. Injured Animals: Horizontal Activity ED 17

Horizontal Activity: Enrichment Day 17. See Figure 46. Data from 2 animals out of a total of 98 were not used (1 male PE; 1 female SE) because of complications resulting from surgery and injury. When all animals were considered together, females were more active than males [Gender: F (1, 88) = 8.79, p <0.05]. Isolated animals were more active than socially-enriched animals [Social: F (1, 88) = 17.82, <0.001] and physically-enriched animals were less active than non-physically-enriched animals [Physical: F (1, 88) = 7.56), p <0.05]. When the sexes were examined separately, the effects of social and physical enrichment were present for males and females. For males, isolated animals were more active than socially-enriched animals [Social: F (1, 43) = 19.28, p <0.001]. These effects of social enrichment in males accounted for 31% of activity variance. The effects of physical enrichment on activity remained, but existed only as a trend [Physical: F (1, 43) = 3.09, p =0.086], accounting for 6.7% of activity variance. For females, both social

[Social: F (1, 45) = 5.72, p <0.05] and physical [Physical: F (1, 45) = 4.81, p <0.05] enrichment reduced activity. Again, the effects of social enrichment accounted for a greater proportion of activity variance (11.3%) compared to the effects of physical enrichment (9.6%).

Horizontal Activity Enrichment Day 28. See Figure 47. Data from two animals (one male PE, one female SE) out of a total of 98 were not used because the animals died secondary to complications from the injury. When all animals were considered together, females again were more active than males [Gender: F (1, 88) = 17.11, p <0.001] and animals in the isolated groups were more active than the animals in the social groups [Social: F (1, 88) = 6.74, p <0.05]. When data were further analyzed within gender, the pattern of activity for males was similar to day 2 with isolated animals exhibiting greater total activity than the socially-enriched animals [Social: F (1, 43) 9.55, p <0.05]. There were no effects of physical enrichment or social enrichment on activity levels for females.

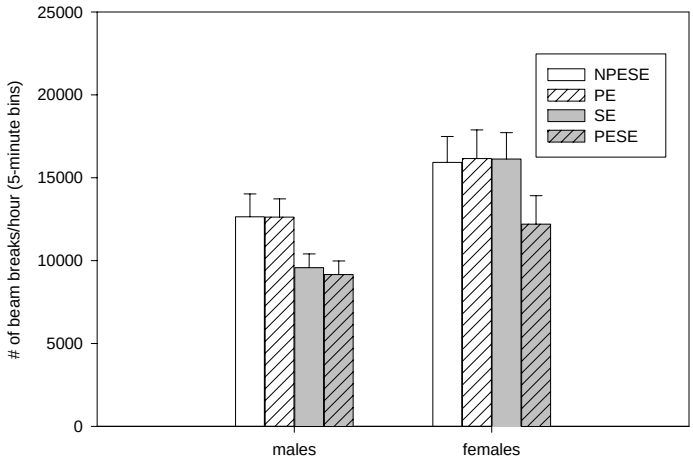

Figure 47. Injured Animals: Horizontal Activity ED 28

Horizontal Activity within Session: Injured Animals

Because there was a pattern for the effects of social enrichment to affect locomotor activity on each day and to parallel the previous data analytic strategy in non-injured animals, within session activity was examined on each day using repeated-measures analyses of variance. As with Experiment I, these analyses were conducted to provide a clearer picture of how activity levels changed across the 60-minute testing session.

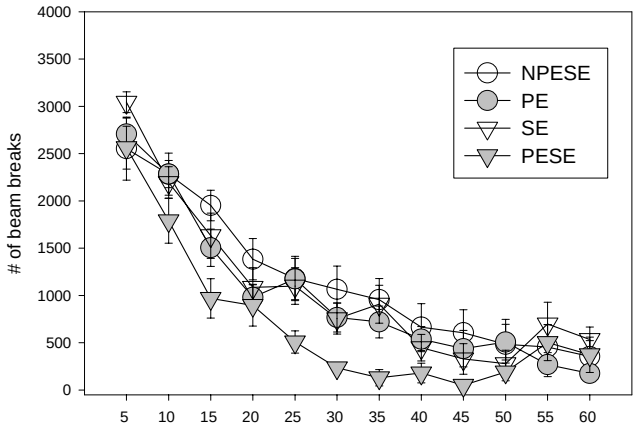

Figure 48. Injured males. Within session horizontal activity ED 12

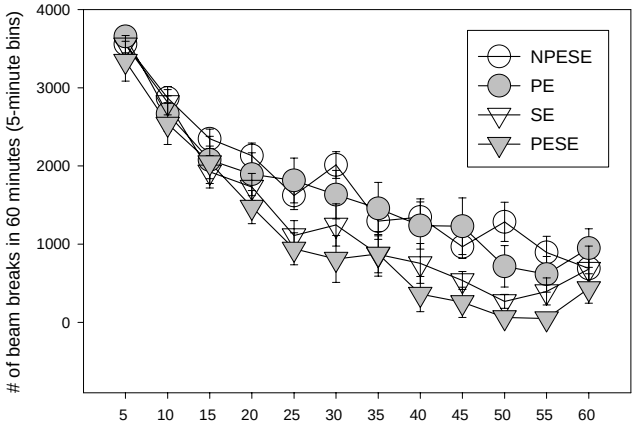

Figure 49. Injured females: Within session horizontal activity ED 12

Within-session analyses

Enrichment Day 12. See Figures 48-49.

On enrichment day 12, when all animals were analyzed together, there was a significant effect of Time with all groups exhibiting a decrease in activity across the testing situation [Time: $F(11, 902) = 210.29$, $p < 0.001$]. Females were more active than males [Gender: $F(1, 82) = 23.93$, $p < 0.001$]. Isolated animals were more active than socially-reared animals [Social: $F(1, 82) = 12.70$, $p < 0.001$] and non-physically-enriched animals were more active than physically-enriched animals [Physical: $F(1, 82) = 4.89$, $p < 0.05$] throughout most of the session. Physical

enrichment had the greatest effect to reduce activity in males [F (1, 43) = 4.91, p <0.05] and social enrichment had the greatest effect to reduce activity in females [F (1, 39) = 10.71, p <0.05]. Both males [Time: F (1, 473) = 104.13, p<0.001] and females [Time: F (1, 429) = 106.74, p =<0.001] exhibited decreasing activity (*i.e.*, increasing habituation) over time.

Enrichment day 17. See Figures 50-51.

On enrichment day 17, when all animals were analyzed together, there was a significant effect of Time with all groups exhibiting a decrease in activity across the testing situation [Time: F (11, 968) =247.60, p <0.001]. Females were more active than males [Gender: F (1, 88) =8.92, p <0.05]. Isolated animals were more active than socially-reared animals [Social: F (1, 88) = 17.72, p <0.001] and non-physically-enriched animals were more active than physically-enriched animals [Physical: F (1, 88) = 7.67, p <0.05] throughout most of the session.

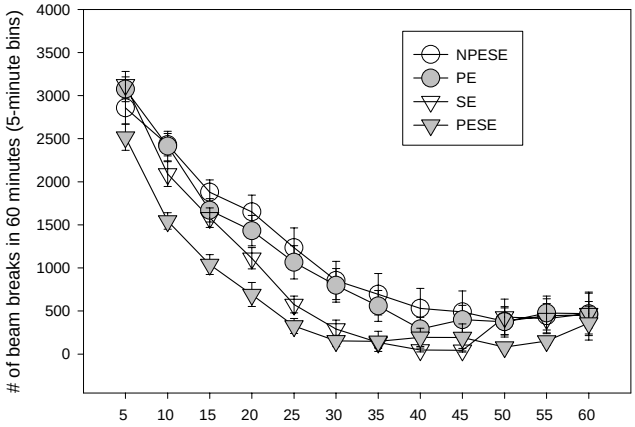

Figure 50. Injured males. Within session horizontal activity on ED 17

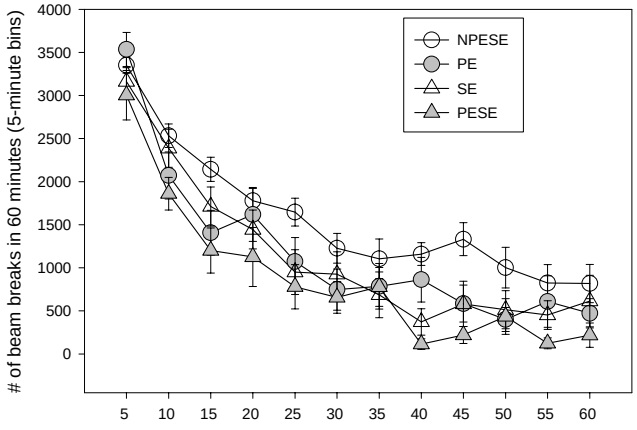

Figure 51. Injured females. Within session horizontal activity on ED 17

Social enrichment had the greatest effect to reduce activity in males [Social: F (1, 43) = 19.19, p = <0.001] with a trend for physical enrichment to reduce activity. For females, both social [F (1, 45) = 5.72, p <0.05] and physical enrichment [F (1, 45) = 4.81, p <0.05] reduced activity across most of the session

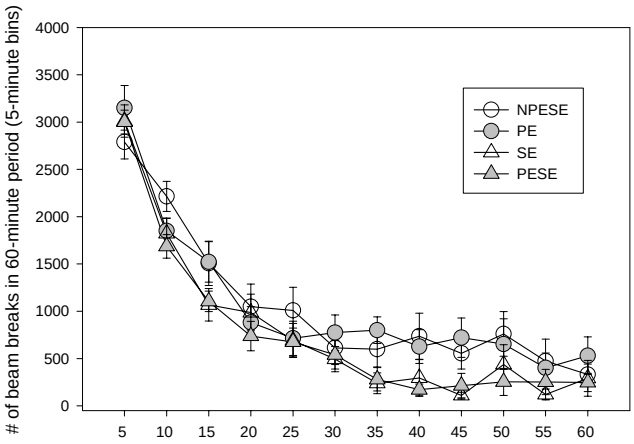

Figure 52. Injured males: Within session horizontal activity on ED 28

Enrichment day 28. See Figures 52-53.

On enrichment day 28, when all animals were analyzed together, there was a significant effect of Time with all groups exhibiting a decrease in activity across the testing situation [Time: F (11, 968) =202.94, p <0.001]. Females were more active than

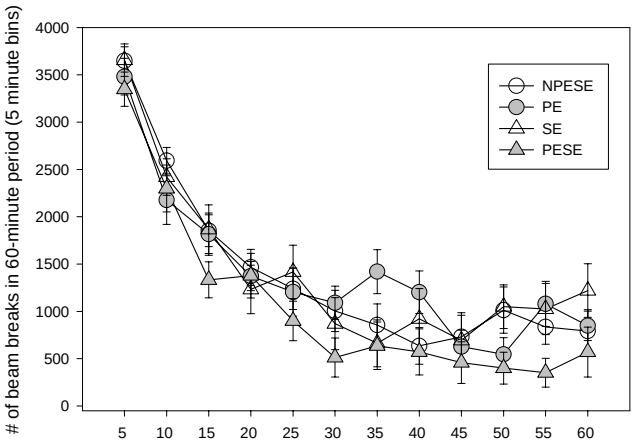

Figure 53. Injured females: Within session horizontal activity on ED 28

males [Gender: F (1, 88) =21.28, p <0.001]. Isolated animals were more active than socially-reared animals [Social: F (1, 88) = 6.01, p <0.05] throughout most of the session. Social enrichment had the greatest effect to reduce activity in males [Social: F (1, 43) = 9.55, p <0.001]. For females, there were no effects of enrichment on activity within the session on ED 28.

Locomotor Summary for Injured Animals

Whether social or physical enrichment altered locomotor habituation depended on time in enrichment (*i.e*., day of measurement) and animal gender. As with intact males, for injured males, the effects of the physical enrichment appeared most important initially, reducing activity on ED 12 and to a lesser extent on ED 17. In contrast to intact males, the effects of social enrichment on activity for injured males did not appear until ED 17. Notably, when the effects of social enrichment did appear, the effect size for social enrichment was robust, accounting for 30% of activity variance on ED 17 and 18% of activity variance on ED 27. The effects of enrichment for injured females, in contrast, followed a pattern more similar to intact males in that the effects of social enrichment appeared early (ED 12), persisted until enrichment day 17, and then disappeared. The effects of physical enrichment, which were present on enrichment day 17 for both injured males and females, were small compared to the effects of social enrichment.

Overall, these results suggest that brain-injured male and female rats vary in their responses to enrichment. Male rats that are brain injured exhibit an early response to physical enrichment but they are most responsive to social aspects of the environment in the long run. Female rats also appear more sensitive to social enrichment than to physical enrichment; however, these effects become less significant over time and are not as robust as are the effects in males over time. As with intact animals, then, the next question is do males and females also vary in their responses to more complex cognitive tasks? That is, are the gender and timing differences in the effects of social and physical enrichment specific to more basic

cognitive domains or does social enrichment remain the most critical part of enrichment as it does for brain-injured animals?

Acoustic startle reflex (ASR) with and without pre-pulse inhibition (PPI)

The acoustic startle reflex (ASR) is an index of reactivity to a sudden unexpected acoustic stimulus. The reflex is altered in response to various neurological states. Only a few studies have examined the effects of traumatic brain injury on the acoustic startle response (Wiley *et al*., 1996; Lu *et al*., 2003) and found that brain-injured animals have less startle amplitude compared to controls. Pre-pulse inhibition (PPI) is the process by which startle is reduced in amplitude if it is preceded by a softer tone presented at a pre-conscious (outside of awareness) level. To date, no studies have examined the effects of brain injury on %PPI. Acoustic startle and PPI were measured as part of Experiment II to parallel the procedures used in Experiment I and to determine if social and physical enrichment altered startle and PPI in brain-injured animals. The procedures used for this measure were the same as those used in Experiment I. The data analytic strategy, therefore, is the same as that used in Experiment I, unless otherwise indicated.

***Analytic approach*.** ASR and % PPI data (*i.e*., startle to 120 dB stimuli and the percentage of pre-pulse inhibition to the stimulus when paired with a 75 dB or 82 dB acoustic pre-pulse or visual pre-pulse) were first analyzed using repeated-measures analyses of variance to evaluate possible changes in startle or pre-pulse inhibition across the enrichment period. Percent PPI was calculated according to the formula: [(Startle amplitude to stimulus without pre-pulse minus startle amplitude

to the same stimulus when paired with a pre-pulse/startle amplitude to a stimulus without pre-pulse)] X 100. Greater startle amplitudes reflect greater reactivity. Greater percent prepulse inhibition reflects greater information processing/sensory gating. Baseline analyses were performed using multivariate analyses of variance (MANOVAS) to determine if groups differed in ASR or PPI prior to manipulation of the variables. Because there were no between-group differences in baseline values, baseline values were not covaried in subsequent analyses.

Baseline analyses. Multivariate analysis of variance (MANOVA) was performed on baseline startle and % PPI (75 dB, 82 dB, and visual) values. Males and females did not differ significantly in their response to the startle stimulus. However, to parallel the previous data analytic strategy, data were analyzed and presented separately for males and females. There were no differences in % PPI among the animals assigned to the different enrichment groups.

Startle Amplitude across the enrichment period.

Repeated-measures ANOVAs were used to evaluate changes in performance across the enrichment period on startle and % PPI values. Changes in startle amplitude across the enrichment period were analyzed first. To parallel the previous data analytic strategy (Experiment I), data were analyzed separately for males and females. Males exhibited increasing startle over time, suggesting increased sensitization to the testing environment [Time: $F(3, 129) = 57.31$, $p < 0.001$). Social and Physical enrichment had no effect on startle amplitude in males. For females, startle also increased significantly over time [Time: $F(3, 129) = 9.17$, $p < 0.001$].

Females in physically-enriched environments startled less overall than animals in non-physically enriched environments [Physical: $F(1, 43) = 6.38$, $p < 0.05$].

% PPI-82 dB over time.

Repeated measures ANOVAs were used to evaluate changes in % PPI across time. Changes in % PPI-82 dB were analyzed first. To parallel the previous data analytic strategies, data were analyzed separately for males and females. Because there were no group differences on % PPI at baseline, baseline values were not covaried in the overall analyses. For males, but not for females, % PPI-82 increased significantly over time [$F(3, 132) = 3.37$, $p < 0.05$]. There were no overall effects of physical or social enrichment on % PPI-82 dB for either males or females.

% PPI-75 dB over time.

Changes in % PPI-75 db level were analyzed next. As with startle amplitude and % PPI-82 dB, data were analyzed separately for males and females. There were no between-group differences in baseline values; therefore, baseline values not covaried in the overall analyses. There were no effects of Time or Enrichment on % PPI-75 for either males or females.

% PPI-visual over time.

Changes in visual PPI were analyzed next. As with startle amplitude and the acoustic % PPI data, data were analyzed separately for males and females. Percent visual PPI did not change over time for either males or females. For males, the effects of social and physical enrichment on % visual PPI interacted and existed as a trend such that the presence of physical objects reduced % visual PPI in the isolated environment, but increased % visual PPI in the social environment [$F(1, 43)$

= 3.85, p =0.056]. There were no effects of physical or social enrichment on % visual PPI in females. Because there were no significant effects of social or physical enrichment on any of the % PPI parameters, univariate analyses were not performed on each day.

ASR Summary

There were no significant effects of enrichment on startle amplitude or % PPI for brain-injured animals.

Passive Avoidance Performance

Because of the robust effect of social enrichment on passive avoidance performance in intact females (Experiment I), all animals in Experiment II participated in this measure of simple memory. Passive avoidance also was chosen in Experiment II to provide an intermediate measure of cognitive performance with a level of complexity between non-conscious ASR and PPI responses, simple information processing as measured by locomotor habituation, and spatial memory as measured by the Morris water maze. The procedures used in Experiment II were the same as those used in Experiment I. Specifically, on the training day (ED 43) each animal was placed into one chamber of the shuttlebox. After an acclimation period, the light went on, and the door opposite, the still-dark chamber opened. When the animals crossed into the dark component, a mild footshock (0.40 mA) was delivered through the grid floor. Twenty-four hours later, animals were tested (ED 44) using the same procedure; however no shock was delivered if animals cross into the darkened chamber. Longer latencies to cross into the dark chamber on the

testing day are interpreted as behavioral evidence of memory (*i.e*., the animals remembers the shock from the previous day). Longer latencies to cross or not crossing into the chamber at all on the second (testing) day indicate better memory function.

Analytic Approach

Data from 10 animals out of a total of 97 were not used. Three of these animals died from undetermined complications resulting from the head injury or the head injury procedure (1 female PE; 1 male SE; 1 female SE). Data from the remaining animals were not valid because of equipment or software failure (1 male, 1 female NPESE; 1 female SE; 3 male, 1 female PESE). Training latencies were compared with testing latencies using Wilcoxon-Signed Ranks Tests (nonparametric t-tests) because latencies did not meet parametric test criteria (*i.e*., homogeneity of variance, normal distribution). Because latency data were bounded (a maximum value of 300 seconds) and did not meet criteria for parametric tests (*i.e*., unequal normal curve distribution), training and testing latencies were analyzed with Kruskal-Wallis nonparametric tests. Finally, because maximal memory for the aversive event is indicated by the animal not crossing into the darkened chamber at all, testing latencies also were recoded into a binary format in which each animal's performance was scored as "crossed" or " did not cross." Then, these data were analyzed with chi-squares to determine whether the proportion of animals that did not cross was significantly greater than chance for specific groups and subgroups.

Task-validity. *See Figures 54-55.* Before pursuing between-subject analyses, training latencies were compared with testing latencies to validate that learning had occurred. That is, did animals demonstrate memory for the aversive event that had occurred 24 hours earlier in the dark chamber by taking longer to cross into the dark chamber on the testing day? Testing latencies were significantly longer than training latencies when all subjects were considered together ($Z = -7.61$, $p < 0.001$) as well as for subgroups indicating that memory had occurred.

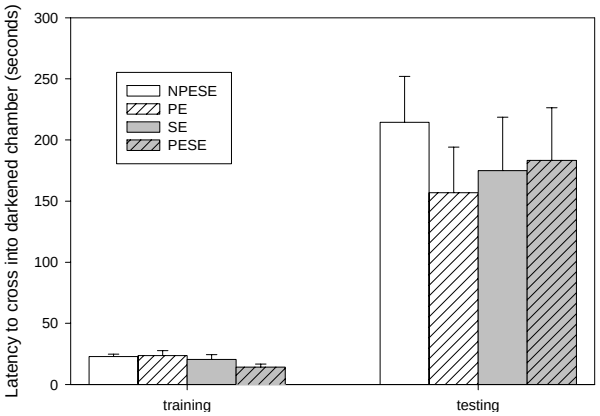

Figure 54. Injured males. Passive avoidance training and testing latencies

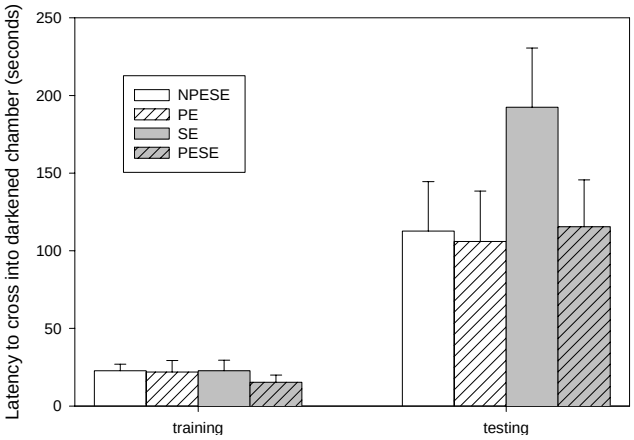

Figure 55. Injured females. Average passive avoidance training and testing latencies

***Training latencies*.** *See Figure 56*. Training latencies were examined non-parametrically using Wilcoxon-Signed Ranks because data did not meet criteria for parametric testing (normal distribution, homogeneity of variance).

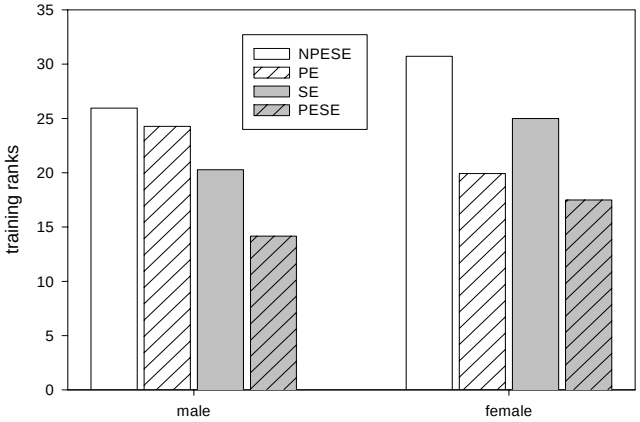

Figure 56. Injured animals. Mean rank scores of passive avoidance training latencies

When all animals were analyzed together, there was a trend for males to have longer latencies to cross into the darkened chamber than females (X= 2.83, df = 1, p =0.092). Both socially (X = 5.01, df =1, p <0.05) and physically enriched animals (X =4.89, df = 1, p <0.05) had shorter latencies to cross into the darkened chamber than did isolated and non-physically enriched animals. When data were further analyzed within gender, for males, socially-enriched animals had shorter training latencies than did isolated animals [X = 4.01, df = 1, p <0.05]. In contrast, for females, there was a trend for animals reared in physically-enriched environments to have shorter latencies than animals reared in isolated environments (X = 5.74, df = 1, p <0.05).

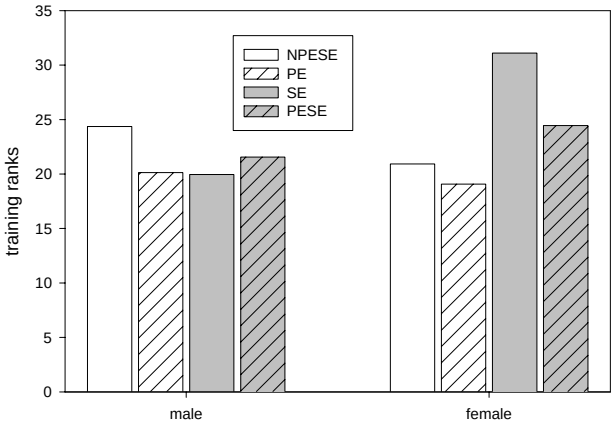

Figure 57. Injured animals: Mean ranks of passive avoidance testing latencies

Testing latencies*. See Figure 57.* Testing latencies were examined non-parametrically using Wilcoxon-Signed Ranks because data did not meet criteria for parametric testing (normal distribution, homogeneity of variance). Latency on the testing day is interpreted as evidence of memory. Animals that took longer to cross or that did not cross into the darkened chamber are said to have better memories than animals that crossed quickly to the other side. When all animals were analyzed together, there was a trend for males to have longer latencies to cross than females (X = 2.80, df = 1, p =0.094). When data were further analyzed within gender, there were no effects of physical or social enrichment on latencies to cross

for males. In contrast, for females there was a trend for animals reared in socially-enriched environments to have longer latencies to cross than isolated animals (X = 3.69 df = 1, p = 0.055).

***Chi-Squares*.** *See Table 5.* Because maximal memory for the task is indicated by the animal not crossing into the darkened chamber at all, proportions of animals not crossing (*i.e*., animals that remembered) were compared with the proportions of animals crossing (*i.e*., animals that did not remember). When all animals were considered together, more animals did not remember (crossed) than remembered (did not cross). This difference was primarily the result of poor female performance. Within males, there were no differences in number of animals that crossed vs. animals that did not cross. In contrast, among females, more animals crossed than did not cross.

Because there was a trend for males and females to differ in their performance on this task, males and females were examined separately. Among males there were no differences in the number of animals that crossed (*i.e*., did not remember) vs. did not cross (*i.e*., remembered), and this performance remained consistent regardless of enrichment status with enrichment neither improving nor impairing memory on this task. Among females, however, comparisons within specific subgroups indicated that significantly more animals crossed than did not cross in each subgroup except for the SE (social only) group.

Table 5. Experiment II: Number of animals that did not remember (*i.e.*, crossed into the dark chamber) vs. remembered (*i.e.*, did not cross) on the testing day.

Group Tested	**# crossed**	**# did not cross**	**Chi-square(df)**	**p value**
All animals	56	32	6.545 (1, 88)	$p < 0.05$
Males	21	21	0.000 (1, 42)	$p = 1.000$
Females	35	11	12.522 (1, 46)	$p < 0.001$
Male-NPESE	4	7	0.818 (1, 11)	$p = 0.366$
Male-PE	7	4	0.818 (1, 11)	$p = 0.366$
Male-SE	5	6	0.910 (1, 11)	$p = 0.763$
Male-PESE	5	4	0.111 (1, 9)	$p = 0.739$
Female-N PESE	11	2	6.231 (1, 13)	$p < 0.05$
Female-PE	10	2	5.333 (1, 12)	$p = 0.021$
Female-SE	5	5	0.000 (1, 10)	$p = 1.000$
Female-PESE	9	2	4.455 (1, 11)	$p < 0.05$

Passive Avoidance Summary. Overall, males performed better on this simple memory task than did females. Enrichment did not alter performance of males. Females, in contrast, generally did not perform as well on this task when compared to males. However, socially enriched females had longer latencies to cross into the darkened chamber on the testing day when compared to isolated females. Further, the social only group was the only group in which the number of animals that crossed was not greater than the number of animals that did not cross, suggesting that the social enrichment environment may have helped to improve memory in brain-injured females.

Morris Water Maze

The Morris water maze is a complex task that was used in this experiment (enrichment days 22-26) to index spatial learning and spatial memory. The water

maze task procedures used in Experiment II were the same as the procedures used in Experiment I and were based on procedures previously used in the enrichment literature. The task was conducted across 5 days with 4 trials on each day. Three minutes separated each trial. The platform remained in the same position each day, but the release point of the animals varied across each trial. Because the platform remained in the same location for the duration of the experiment, Trial 1 of each day was used to index long-term memory.

Analytic Approach

As with experiment I, learning was assumed to have occurred if, over several trials, animals swam more quickly and directly to the visible platform. Paired t-tests comparing average Trial 1 latency and distance to Trial 4 latency and distance were used to establish task validity. That is, performance on the last trial of each day should be better than the first trial of each day if animals learn where the platform is located.

Evidence that animals have transformed the location of the platform into long-term memory is provided by examining latencies to the platform on the first trial of each day (maze days 2 -4). Latency to find the platform on the first trial of day 1 was not included in these analyses because it was the first water maze exposure for all animals. Shorter latencies to find the platform on the first trial of each day are evidence that the animals have retained the position of the platform in long-term memory store (between days; 24-hours). Distance to find the platform on Trial 1 also should decrease over time as the animals learn that the position of the platform does not change. Repeated analyses of variance were used to examine change in

Trial 1 performance across the 4 days of testing and to determine if changes over time differed depending on animal group.

Finally, to determine whether overall performance increased over the 5 days, repeated measures analyses were used to examine changes in mean latency to find the platform and mean distance traveled to the platform across days 1-5 (ED 22-26). To parallel the previous data analytic strategies, group difference on mean performance (latency and distance to the platform) were examined on each day.

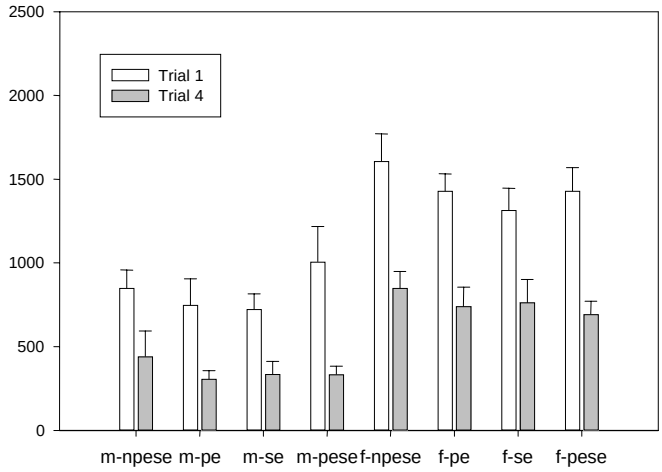

Figure 58. Injured animals: Distances traveled on Trial 1 and Trial 4 averaged across ED 22-26

Task Validation. *See Figure 58.* When all animals were analyzed together, paired t-tests indicated that performance improved from Trial 1 to Trial 4 for all animals (latency: $t = 15.64$, $df = 95$, $p < 0.001$; distance: $t = 10.69$, $df = 95$, $p < 0.001$). These effects remained when analyses were split further by gender and group. The results from the separate t-tests for latency and distance are presented in Appendix C.

Water Maze Performance Across Enrichment Days. *See Figures 59-60.*

Repeated-measures ANOVAs were used to examine changes in mean latency and distance to find the platform across the 5 testing days (ED 22- ED 26). All animals reliably learned the hidden platform within the 5 days of training in the Morris water maze. When all animals were analyzed together, there was a main effect for Time such that the mean latency [F (4, 352) =65.97, p <0.001] and the distance traveled [F (4, 328) =21.70, p <0.001] to find the platform decreased from Day 1 to Day 5, suggesting that the animals were finding the platform faster and learning a more direct route to the platform over time. Males had shorter latencies [F (1, 88) = 10.06, p <0.05] and swam shorter distances [F (1, 88) = 81.99, p <0.001] than did females. There also were trends for physically enriched [F (1, 88) = 2.97, p =0.088] and socially enriched [F (1, 88) = 3.01, p =0.086] animals to have shorter latencies to find the platform than non-physically and non-socially enriched animals. Because there was a main effect for gender, the effects of social and physical enrichment also were evaluated separately for males

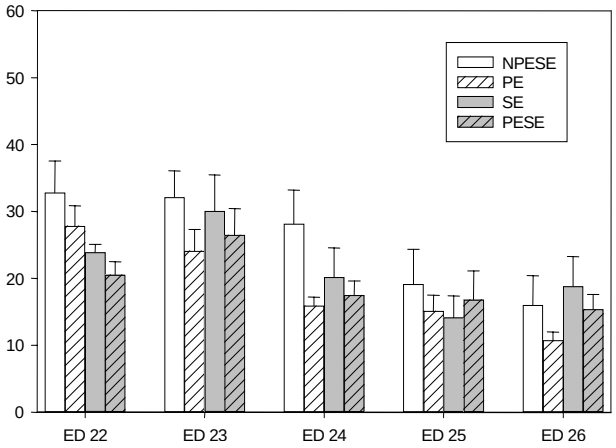

Figure 59. Injured males: Mean latency to reach platform on ED 22-26

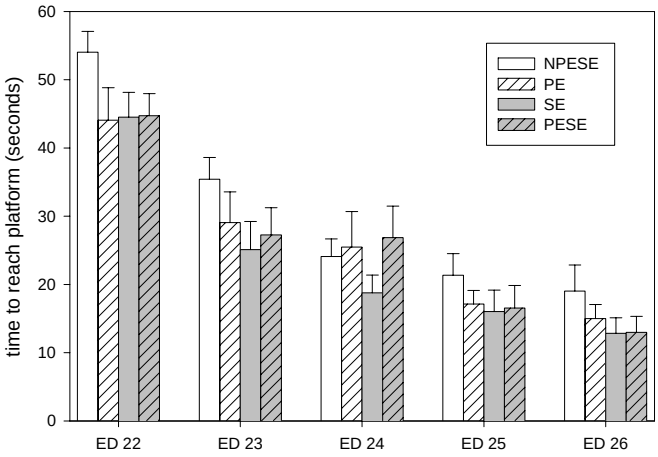

Figure 60. Injured females: Mean latency to reach platform on ED 22-26

and females. For males, the effects of physical enrichment to improve performance existed as a trend [F (1, 45) = 3.53, p =0.080]. For females, there was a trend for social but not physical [F (1, 45) = 3.35, p =0.074] enrichment to improve latency to find the platform over time.

Next, because there was an effect for time and to parallel the previous data analytic strategy, the separate effects of physical and social enrichment were examined on each treatment day. Only those days in which a significant effect was found are presented. However, F values, and p values for the non-significant days are presented in Appendix C.

Water Maze Day 1 (ED 22) Performance.

For males, socially-enriched animals (PESE and SE) had shorter latencies to find the platform than did isolated animals F (1, 43) =7.19, $p < 0.01$). Animals reared in the PESE group had the shortest latencies overall. There was no effect of social or physical enrichment on latency to reach the platform for females.

Water Maze Day 3 (ED 24) Performance.

Within males, animals reared in physically-enriched environments (PESE and PE) had shorter latencies to find the platform than animals in non-physically enriched environments [F (1, 92) =4.15, $p <0.05$). Animals reared in the PE group had the shortest latencies overall. There was no effect of social or physical enrichment on latency to reach the platform or distance traveled for females.

There were no effects of social or physical enrichment on Morris water maze performance for ED 23, 25, and 26.

Long Term Memory: Performance on Trial 1.

There were no effects of enrichment on long-term memory performance as measured by performance on Trial 1 for either males or females.

Chi square analysis comparing good and poor performers.

Because distances and latencies to reach platform vary considerably within groups, latencies to find the platform also were analyzed in a binary format with data coded based on a median split of mean performance times across days. Animals that found the platform in less than the median time were considered "good performers," whereas animals that found the platform in more than the median time were considered "poor performers." When all animals were considered together, males were better at the task (# good > # poor) than were females (# poor > # good). Comparisons within specific subgroups indicated that for males both social and physical enrichment had a beneficial impact on performance. Specifically, males in the PE, SE, and PESE had a somewhat greater number of "good" performers than "poor" performers. Performances did not differ in isolated groups. For females, the pattern was slightly different in that the isolated environment (N-PESE) appeared to have deleterious effects on performance with a greater number of poor performers than good performers in the NPESE and PE groups. The social and physical environment appeared to buffer these effects slightly in that, within the SE and PESE groups, there were no significant differences between good performers and poor performers (*i.e.,* the number of poor performers did not outnumber the number of good performers).

Table 6. Experiment II: Number of good performers (*i.e.*, <23.02 seconds to find platform) vs. poor performers (> 23.02 seconds) based on grand mean across days.

Group Tested	**"Good" Performers**	**"Poor" Performers**	**Chi-square(df)**	**p value**
All males	33	14	7.651 (1)	$p = 0.564$
Males-NPESE	5	7	0.333 (1)	$p = 0.683$
Males-PE	10	1	7.634 (1)	$p < 0.05$
Males-SE	9	3	3.000 (1)	$p = 0.083$
Males-PESE	9	3	3.000 (1)	$p = 0.083$
All females	15	34	7.367 (1)	$p < 0.05$
Female-NPESE	2	12	7.143(1)	$p < 0.05$
Female-PE	3	9	3.000 (1)	$p = 0.083$
Female-SE	5	6	0.091 (1)	$p = 0.763$
Female-PESE	5	7	0.333 (1)	$p = 0.564$

Morris Water Maze Summary. All groups within both males and females exhibited improved performance over trials 1-4 and over days 1-5, suggesting that all animals were learning the position of the maze and retaining it in immediate or short-term memory. Males were the most sensitive to the effects of enrichment exhibiting effects of enrichment on day 22 and 24. As with intact animals, the superior performance of the enriched animals appeared early, then the non-enriched animals caught up. Females, in contrast exhibited little response to enrichment during this time period.

Overall, the results of this analysis suggest that males are more sensitive to the effects of physical and social enrichment than are females on this task. In fact, enriched brain-injured females exhibited minimal evidence of superior performance when compared to non-enriched females. These findings are in contrast to those found in intact animals in which females did respond to the effects of social enrichment, but these effects occurred later and were not as robust as those found in males.

CONFIRMATION OF HYPOTHESIS

Experiment I

Hypothesis 1

The hypothesis that environmentally enriched (PE, SE, or PESE) animals will exhibit superior performance on all tasks and measures (*i.e.*, increase habituation in open-field activity, increase habituation in ASR, increase PPI, enhance passive avoidance performance, enhance Morris water maze performance) when compared to non-enriched (isolated) animals was **partially supported.**

Results

Socially and physically enriched-rats exhibit enhanced performance compared with non-enriched rats (isolated) on measures of simple information processing (*i.e.,* locomotion), simple working memory (*i.e.*, passive avoidance), and spatial memory and learning (*i.e*., Morris water maze), replicating and extending past work examining the effect of enrichment on these measures (Gardner, Boitano, Mancino, & D' Amico, 1975; Smith, 1972; Pham *et al.,* 1999; Varty *et al.,* 2000). Social or physical enrichment did not enhance acoustic startle or %PPI, another index of simple information processing or sensory gating.

Effects of social enrichment were greater than the effects of physical enrichment and greater (*i.e*., larger effect sizes, more consistent effects) for males than for females. The presence of a larger effect for social enrichment and a larger effect of enrichment for males are new findings.

Hypothesis 2

The hypothesis that performance on simpler measures of cognitive functioning (*i.e.,* open-field activity, acoustic startle activity, pre-pulse inhibition) will be associated with performance on more complex measures (*i.e.,* Morris water maze) was **partially supported.**

Results

Social enrichment was the key factor affecting the performance of males on locomotor habituation, a simple information-processing task. Social enrichment also was the key factor enhancing performance on the Morris water maze task, a complex spatial memory task that is dependent on attention and information processing. Impaired performance on the simple attention task (*i.e*., % PPI) was not related to subsequent performance on the Morris water maze task. For females, impairments in %PPI was not related to performance on more complex measures, including passive avoidance, a simple working memory task, or the Morris water maze, a complex spatial memory task.

Hypothesis 3

The hypothesis that for male subjects, the effects of enrichment to enhance cognitive performance will be: PESE > SE = PE > N-PESE was **not supported.**

Results

The combined socially and physically enriched environment did not consistently prove to be better than the other treatment groups. Instead, the order of effects varied depending on specific task and time in enrichment. The current study is slightly inconsistent with previous work that reports that the combined enrichment

study (PESE) has the greatest effect to enhance cognitive performance (Gardner, Boitano, Mancino, & D' Amico, 1975; Smith, 1972; Varty *et al*., 2000). In this study, although the NPESE group exhibited the poorest performance on measures of simple information processing (*i.e.,* locomotion) and complex spatial memory (*i.e*., Morris water maze); the SE group exhibited the poorest performance on a measure of simple attention or sensory gating (% PPI). When the effects of social and physical enrichment were examined separately, social enrichment appeared to have a greater effect than physical enrichment to improve performance on locomotion, passive avoidance, and Morris water maze.

These results extend previous findings by suggesting that for males, social enrichment may be the key to improved performance on selected measures and that PE has minimal effects. Possible explanations for the ASR/PPI findings are addressed in the discussion.

Hypothesis 4

The hypothesis that for female subjects, the effects of enrichment to enhance cognitive performance will be: SE $\geq$ PESE > PE = NPESE was **not supported.**

Results

As with males, for females, the effects of social and physical enrichment depended on the task. Only a few studies have examined the effects of enrichment on cognitive performance in female rats. Previous studies have yielded findings similar to those reported in males in that the combined enrichment study (PESE) appears to have the greatest effect to enhance cognitive performance. In this study,

the PE group exhibited the poorest performance on measures of simple information processing (i.e., locomotion), but physical enrichment enhanced performances on measures of attention (*i.e*., PPI) and complex spatial memory (*i.e.,* Morris water maze). Social enrichment also helped to improve performance on the Morris water maze and the Passive avoidance task.

These results extend previous findings by suggesting that the effects of enrichment for females may depend on task demands. Possible explanations for these results are addressed in the discussion.

Experiment II

Hypothesis 1

The hypothesis that environmentally enriched (PE, SE, or PESE) animals will exhibit superior performance on all tasks and measures (*i.e.*, increase habituation in open-field activity, increase habituation in ASR, increase PPI, enhance passive avoidance performance, enhance Morris water maze performance) when compared to non-enriched (isolated) animals was **partially supported.**

Results

The effects of enrichment to enhance performance depended on animal gender and the specific cognitive task. Enriched-rats exhibit enhanced learning compared with non-enriched rats on measures of simple information processing (*i.e*., locomotion), simple working memory (*i.e*., passive avoidance), and spatial memory and learning (*i.e*., Morris water maze), replicating and extending past work that brain-injured rats recovering in enriched environments exhibit enhanced cognitive performance compared with brain-injured rats recovering in non-enriched conditions (Passineau *et al.,* 2001; Ohlsson & Johansson, 1995). Enrichment did not enhance acoustic startle or %PPI, another index or simple information processing or sensory gating.

Effects of social enrichment were generally greater than the effects of physical enrichment and more robust for males than for females. The presence of a larger effect for social enrichment and for males are new findings.

Hypothesis 2

The hypothesis that performance on simpler measures of cognitive functioning (*i.e.,* open-field activity, acoustic startle activity, pre-pulse inhibition) will be associated with performance on more complex measures (*i.e.,* Morris water maze) was **partially supported.**

Results

Social and physical enrichment enhanced the performance of males on locomotor habituation, a simple information processing task, and on the Morris water maze task, a complex spatial memory task that is dependent on attention and information processing. Impaired performance on the simple attention task (*i.e.,* % PPI) was not associated with subsequent performance on the Morris water maze task (*i.e*., poor performance on simpler measures did not = poor performance on more complex measures). For females, impairments in %PPI did not predict poor performance on more complex measures, including passive avoidance, a simple working memory task, or the Morris water maze, a complex spatial memory task.

Hypothesis 3

The hypothesis that for male subjects, the effects of enrichment to enhance cognitive performance will be: PESE > SE = PE > N-PESE was **not supported.**

Results

The combined socially and physically enriched environment did not prove to be consistently better than the other treatment groups. The current study contrasts slightly with previous work in that previous studies have reported that the combined enriched environment (PESE) has the greatest effect to enhance cognitive

performance in brain-injured rats (Passineau *et al.,* 2001; Ohlsson & Johansson, 1995).

In this study, although the NPESE group exhibited the poorest performance on measures of simple information processing (*i.e*., locomotion) and complex spatial memory (*i.e.,* Morris water maze), enrichment had no effect on a measure of sensory gating/attention (*i.e*., %PPI) or simple working memory (*i.e*., passive avoidance). Further, the findings for enrichment to improve performance on the Morris water maze did not appear as robust as the findings reported in previous studies. Possible explanations for the ASR/PPI and passive avoidance results as well as possible explanations for the weak Morris water maze findings are addressed in the discussion.

Hypothesis 4

The hypothesis that for female subjects, the effects of enrichment to enhance cognitive performance will be: SE $\geq$ PESE > PE = NPESE was **not supported.**

Results

For females, as with males, the effects of social and physical enrichment depended on the task. Only one study to date has examined the effects of enrichment on cognitive performance in female brain-injured rats (Wagner *et al*., 2002). Findings from this study suggest that brain-injured females exhibit little response to the effects of enrichment on Morris water maze performance following cortical impact injury. In the current study, the PE group exhibited the poorest performance on measures of simple information processing (*i.e*., locomotion), but physical enrichment enhanced performances on measures of attention (*i.e*., PPI)

and complex spatial memory *(i.e*., Morris water maze) and social enrichment improved simple information processing (*i.e*., locomotor habituation) and simple working memory (*i.e*., Passive Avoidance). The fact that social enrichment did not enhance performance on the Morris water maze for brain-injured females extends a recent finding (Wagner *et al*., 2002) by suggesting that brain-injured females may be less sensitive than are brain-injured males to the effects of enrichment or that that effects of enrichment for brain-injured females may be task dependent. Possible explanations for these results are addressed in the discussion.

DISCUSSION

The goals of this doctoral research were to examine the separate effects of social enrichment (SE) and physical enrichment (PE) on cognitive performance of neurologically-intact and brain-injured rats and to determine if there were gender differences in these effects. Measures of basic and complex cognitive processing were used to determine if enrichment effects on simpler measures of cognitive performance would predict enrichment effects on more complex cognitive measures.

Two separate experiments were conducted to address these goals. Experiment I compared the effects of N-PESE, PE, SE, and PESE on cognitive performance in neurologically-intact male and female Sprague-Dawley rats. Experiment II compared the effects of NPESE, PE, SE, and PESE on cognitive performance in brain-injured male and female Sprague-Dawley rats. Gender differences were evaluated by comparing the responses of male and female rats. Basic and complex cognitive processes were evaluated by including a wide variety of behavioral measures. Specifically, the behavioral responses measured included a task measuring simple active information processing (*i.e*., locomotion habituation), a task measuring passive information processing (*i.e*., ASR and PPI), a simple working memory task (*i.e.,* passive avoidance), and a complex spatial learning and memory (*i.e.,* Morris water maze) task. These measures were included to provide a comprehensive picture of how enrichment influences recovery across various levels of cognitive complexity.

In particular, the specific aims of this project were to determine: 1) which components of the enriched environment have the greatest influence to enhance

cognitive performance in neurologically-intact animals; 2) whether intact males and females respond differently to specific components of the enriched environment; 3) which components of the enriched environment have the greatest influence to enhance performance of brain-injured animals; 4) whether brain-injured males and females respond differently to the effects of the environmental enrichment.

The major findings of the experiments were that: 1) social enrichment had the greatest effect to improve cognitive performance in neurologically-intact and brain-injured animals; 2) intact males and females responded differently to the effects of social and physical enrichment; 3) brain-injured males and females responded differently to the effects of social and physical enrichment; 4) the effects of enrichment in males and females were task dependent.

The sections below summarize the findings for each independent variable from Experiment I and Experiment II. First, a discussion pertaining to the results from Experiment I is presented. Then, a discussion pertaining to the results from Experiment II is presented. In each discussion section, a table summarizing the major findings from each experiment is presented first. Then, possible explanations for the observed results are discussed. Next, relevant methodological issues and study limitations are addressed. Finally, possible implications for the current results and directions for future studies are discussed.

Table 7. Summary of major findings from Experiment I (Intact Animals)						
	Males Social	**Males Physical**	**Males Social X Physical**	**Females Social**	**Females Physical**	**Females Social X Physical**
Locomotion	**Improved** performance on all days	**Improved** performance on ED 12	No effect	**Improved** performance on ED 12 & 28	No effect	No effect
ASR	No effect	No effect	No effect	No effect	No effect	No effect
% PPI -82	**Decreased *** PPI on ED 15	No effect	**PESE > SE on ED 15 and 20**	No effect	**Increased** PPI on ED 30	**PESE > SE** on ED 15 and ED 30
%-PPI -75	**Decreased** PPI on ED 20	No effect	**PESE > SE on ED 15**	**Decreased** PPI on ED 30	**Increased PPI on ED 15 and 30**	**PESE > SE on ED 30**
%-PPI-visual	No effect	**Decreased PPI on ED 20 and ED 30**	No effect	No effect	No effect	No effect
PA Test				**Improved performance**	No effect	No effect
MWM mean time	**Improved performance** on ED 22-23	No Effect	No effect	No effect	No effect	**Improved performance** on ED 24
MWM Trial I time	**Improved performance**	**Improved performance**	No effect	No effect	No effect	No effect

* It is unclear whether decreased PPI represents an improvement in performance or a decrement in performance. Therefore, these results are presented in the table without interpretation.

Experiment 1: Locomotion

The separate effects of social vs. physical enrichment on locomotor habituation (*i.e.*, decreased activity) depended on time in enrichment (*i.e.,* day of measurement) and animal gender. For males, isolation-reared rats (*i.e.*, NPESE and PE) were more active in the open field compared to socially-reared rats, regardless of physical enrichment. Physical enrichment also reduced activity for intact males, but these effects were less robust and more transient than the effects of social enrichment. Although both physical and social enrichment reduced activity, social enrichment accounted for a greater percentage of activity variance (17.5%) than did physical enrichment (5.6%). Further, the effects of social enrichment were present on enrichment day 12 and persisted until enrichment day 28. The effects of physical enrichment disappeared after ED 12.

Among females, the effects of enrichment were less robust than the effects of enrichment in males. Further, social enrichment, but not physical enrichment increased habituation (*i.e*., reduced activity), suggesting that for females, social enrichment is the key factor affecting simple information processing and that intact females may be insensitive to the effects of physical enrichment on this measure. For females, the effects of social enrichment also appeared to take longer to emerge and did not consistently affect performance. These results suggest that females may take longer to derive consistent benefit from social enrichment. Together with the results from males, these findings suggest that social environment is a key

component of environmental enrichment and that there are gender differences in enrichment effects.

The findings that enriched animals exhibited greater habituation to a novel environment and less hyperactivity in the open field than did isolated animals replicates previous reports (Varty *et al*., 2000; Gardner *et al*., 1975; Einon & Morgan, 1976; Schrijver, Bahr, Weiss, & Wurbel, 2002; Larsson, Winblad, & Mohammed, 2002). The findings that the effects of social enrichment were more robust and persisted longer than the effects of physical enrichment are new.

It is interesting that the effects of physical enrichment were so much less pronounced than the effects of social enrichment. Animals reared in physically-enriched environments are repeatedly exposed to novel environments which they are allowed to explore freely. One might expect, therefore, that physically-enriched animals would be more primed for the exploration of a novel or unfamiliar environment and, therefore, adapt more quickly to a new situation. The current findings suggest that this is not the case. Instead, social enrichment is the key to improve information processing, as evidenced by increased habituation in the open field. Additionally, the current results suggest that a social environment may be a critical element to reduce hyperactivity – a behavior that may impair learning efficiency. The exact mechanisms for the observed effects, including the superior effects of social enrichment, are less clear and merit further study. Future investigations might include neuroanatomical and neurochemical examination of subjects reared with social vs. physical enrichment. Future studies also could

examine how physical and social enrichment affect emotionality in ways that might alter activity in the open field.

Experiment I: Acoustic Startle Response and Prepulse Inhibition

The acoustic startle reflex is an automatic, non-volitional measure that reveals reactivity to a novel startling acoustic stimulus. Pre-pulse inhibition is the process by which a stimulus presented briefly before the startle stimulus reduces reaction to the subsequent stimulus. Prepulse inhibition indexes sensory-gating. Increases in startle without a change in percent pre-pulse or decreases in percent PPI indicate impairments in sensory-gating processing.

In the present experiment, enrichment had no effects on overall startle amplitude for males or females. The effects of enrichment on % PPI varied across the enrichment period and were gender specific. Among males, the effects of enrichment again appeared early, affecting %PPI responses on ED 15 and 20, but not ED 30. Further, males were most responsive to the effects of social enrichment. Contrary to what was expected, social enrichment appeared to impair % PPI as evidenced by lower % PPI levels among socially-enriched animals when compared to isolated animals. The effects of physical enrichment appeared to buffer these effects such that animals in the PESE environment exhibited greater %PPI than animals in the SE environment. Physical enrichment also decreased visual % PPI on ED 20 and 30. In contrast to the apparent negative effects of enrichment on % PPI in males, physical enrichment increased % PPI for females on ED 15 and 30.

The findings in males are in contrast to previous reports of isolation effects on % PPI. Specifically, previous studies have reported that animals reared in isolation exhibit decrements in PPI compared to group-housed or enriched animals (Geyer *et al*., 1993; Bakshi & Geyer, 1999). There are several possibilities that might explain these contrasting results. In previous studies, animals reared in isolation received minimal contact with the experimenter or with other animals, representing a more face valid isolated or impoverished condition. In the current study, animals in the isolated housing condition (N-PESE) did not differ from the other groups in the amount of handling they received from the experimenter and they were housed in the same room as animals in the other conditions. It is possible that the more frequent handling of animals used in the current study attenuated the detrimental effects of an isolated environment on PPI responses.

Another possibility for the unexpected PPI findings may be differences in the timing of isolation or the age at which animals were first exposed to the isolated conditions. Previous studies with rats have indicated that animals reared in isolation exhibit disruption of PPI, characteristic of neurodevelopmental disorders *(i.e.,* schizophrenia). In the majority of these studies, however, isolation rearing referred to a procedure in which animals were housed singly, immediately after weaning (21-28 days after birth) and, therefore, deprived of social contact with their peers during development. In this study, animals were housed in isolation at postnatal day (PND) 45. Isolation-induced deficits are less likely to occur when isolation occurs during adulthood (Wilkinson *et al*., 1994). The current findings, in which no PPI deficits were found in isolated animals, are consistent with previous

reports that there may be a critical developmental period during which isolation has the greatest effect to disrupt PPI (Wilkinson *et al*., 1994).

Why socially-enriched animals exhibited lower % PPI than isolated animals, is not clear, but replicates previous findings in Long-Evans males (Faraday *et al*., 1998). The animals reared in social groups may have experienced greater distress when placed individually in the ASR holders, because experience in the holders represented a greater change from their normal environment. This explanation cannot fully account for the current results, however, because animals were tested individually in all behavioral measures and enhanced performance was found in socially-enriched animals on several of the behavioral tests. Further studies should try to replicate these findings.

Although the findings from ASR and PPI did not support the original hypothesis regarding enrichment effects, there are three important conclusions that can be drawn from Experiment I: 1) the effects of isolation housing on cognitive performance may be task dependent; 2) the potential for isolation housing to disrupt a particular cognitive ability may depend on the critical developmental window specific to that ability; 3) PPI performance can be dissociated from isolation-induced hyperactivity. The effects of enrichment to alter ASR or PPI appear to be dependent on a specific developmental period, but the effects of enrichment on locomotor habituation or hyperactivity appear to be independent of developmental stage. Further, because isolation-induced hyperactivity is present in the absence of % PPI disruptions, hyperactivity cannot account for the increased reactivity that characterizes impairments in prepulse inhibition.

Experiment I: Passive Avoidance

This simple memory task revealed the extent to which animals remembered that they had been previously shocked in a darkened chamber. When 48 neurologically intact females were examined in the passive avoidance chambers, social enrichment again emerged as the key factor affecting performance. Specifically, female rats reared in social environments had significantly longer latencies to cross into the darkened chamber than did isolated animals. These results suggest that socially-enriched females rats had greater memory for the previous aversive event (*i.e*., shock) than did isolation-reared animals.

The finding that enriched female rats perform better on a passive avoidance task than isolated (NPESE) animals replicates past work with male rats (Petit & Alfano, 1979; Crnic, 1983) and previous reports that enrichment improves memory of an aversive event (Woodcock & Richardson, 2000). As with locomotor activity, the passive avoidance results suggest that social enrichment, regardless of physical background, has the greatest effect to enhance cognitive performance, especially on tasks that require the accurate processing of contextual information.

Alternative explanations for the observed effects include differences in locomotor activity (*i.e.,* greater hyperactivity = shorter latencies to cross) or poor inhibitory control among isolated animals. Isolated (*i.e*., N-PESE) animals exhibit greater activity in the open field (Varty *et al*., 2000; Gardner *et al*., 1975; Einon & Morgan, 1976; Schrijver, Bahr, Weiss, & Wurbel, 2002; Larsson, Winblad, &

Mohammed, 2002). Further, isolation housing is associated with changes in prefrontal cortico-striatal monoamine pathways that impair the inhibitory control of behavior (Robbins *et a*l., 1996; Hall; 1998). In this study, the finding that testing but not training latencies differed among groups suggest that poor inhibitory control or hyperactivity cannot fully explain the current findings. Instead, it appears that socially-enriched animals were more sensitive to environmental cues and were better able to transform contextual information into long-term memory store for later use, resulting in greater mean testing latencies.

Experiment I: Morris water maze

This Morris water maze task was included to provide the most complex measure of cognitive performance. Specifically, learning and immediate spatial memory were indexed by how quickly the animal learned the position of the platform. Efficiency in the transformation of spatial information into long-term memory was indexed by how quickly the animals located the position of the platform on the first trial of each day. All animals learned the position of the platform across the 5 testing days.

For males, social enrichment, regardless of physical background, had the greatest effect to improve acquisition of this task with animals reared in socially-enriched environments exhibiting shorter latencies to find the platform compared to animals reared in isolation. Physical enrichment also improved performance, but to a lesser extent than social enrichment. In addition to improving overall acquisition of the task, socially-enriched animals also exhibited faster mean latencies to find the

platform on Trial 1 across successive testing days, suggesting that social enrichment also helps to improve the transformation of spatial knowledge from short-term to long term memory. The findings for male rats replicate previous findings that enriched animals exhibit superior performance on the Morris water maze task compared to non-enriched rats.

For females, physical but not social enrichment improved performance on the Morris water maze, but these effects were more variable and did not persist as long as the effects observed in males. As with locomotor activity, it appears that females may be less sensitive to enrichment effects. Only a few studies have examined the effects of enrichment in female rats (Juraska & Kopcik, 1998; Daniels *et al*., 1999). These studies have reported that female rats exhibit improved performance in response to environmental enrichment, similar to the effects observed in male rats. In the current study, females appeared less sensitive to enrichment effects when compared to males and the effects of enrichment appeared less robust than the effects reported in previous studies.

It is possible that strain differences could explain the different findings. The majority of studies examining the effects of enrichment in female rats have used Long-Evans rats (Juraska & Kopcik, 1998; Daniels *et al*., 1999). The current study used Sprague-Dawley rats. Previous studies have reported strain differences on a variety of behavioral tasks (van der Staay & Blokland, 1996; Lehmann, Pryce, & Feldon, 1999;) and in response to a variety of manipulations (Faraday, 2002; Gleason, Dreiling, & Crawley, 1999). These findings include recent reports of mouse strain differences in response to enrichment (Chapillion, Manneche, Belzung,

& Caston, 1999). Future studies using female rats should include rats of different strains.

Limitations of Experiment I

Overall, the effects of enrichment in intact animals differed depending on animal gender and cognitive task. The effects of social and physical enrichment were task dependent in that enrichment had beneficial effects on simple information processing (*i.e.*, locomotion), simple working memory (*i.e.,* passive avoidance), and complex spatial memory (*i.e.,* Morris water maze), but did not affect general reactivity (ASR amplitude) or sensory gating (% PPI). Enrichment had robust effects in males, but minimal and inconsistent effects in females. While the majority of the findings appear to replicate the previous literature in that enrichment improved performance on a variety of behavioral tasks, the current findings do not appear as marked as those reported in some studies. Two factors that may have contributed to differences in observed effects include: 1) repeated behavioral testing and 2) age of exposure and timing of enrichment.

Repeated Behavioral Testing

Perhaps the greatest factor affecting the current findings (in particular the water maze results) was the fact that repeated tests were conducted on each animal over a relatively brief period of time. Repeated testing on a wide variety of behavioral measures may have acted as a type of enrichment for the isolated animals, providing similar cognitive stimulation to that experienced by the animals reared in socially or physically-enriched environments. The effect of cognitive

stimulation to diminish differences in performance between impoverished and enriched animals has been suggested previously (Larsson *et al*., 2002).

The methods used in the current study were selected to better understand how enrichment affects cognitive processing at different levels of complexity. In an effort to control for these effects, animals were measured only once on each measurement day. Nevertheless, certain interactions could have occurred. For example, the effects of enrichment on Morris water maze were less robust than reported in previous findings. This task was performed at the end of the enrichment period after repeated testing had occurred in the open-field and ASR chambers. The repeated handling associated with these measures or the stimulation resulting from experiencing these tasks may have enhanced subsequent responses in the Morris water maze. Further, the response of females to enrichment on a task measuring simple information processing (*i.e*., locomotor habituation) depended on days since last measurement. That is, females exhibited the greatest response to enrichment on those days in which testing was proceeded by a greater interval of non-testing. This pattern was not present for males, suggesting that females may be more sensitive to interaction of repeated testing and enrichment. Future experiments should space out behavioral measures or include a control group that receives less handling to determine to what extent repeated behavioral testing might attenuate the effects of enrichment.

Timing of enrichment

The less robust findings in the current study also may have resulted from differences in length and timing of enrichment in Experiment I compared to other studies. Cognitive development in animals and humans is influenced by environmental conditions. Animals reared in impoverished or isolated housing conditions exhibit impairments in cognitive and intellectual functioning, whereas animals reared in enriched conditions exhibit enhanced cognitive performance (Gardner *et al*., 1975; Daniel *et al*., 1999; van Praag *et al*., 1999; Schrijver *et al*., 2001). These effects generally are reported to be more marked and persistent when enrichment is introduced early in the organism's life (Kobayashi, Ohashi, & Ando, 2002).

The fact that the effects of enrichment differed depending on a given task suggests that the effect of enrichment on specific cognitive measures also may depend on a specific neurodevelopmental period. For example, the effects of isolation rearing on ASR/PPI recently have been reported to be limited to the pre-pubertal window (Bakshi & Geyer, 1999). The effects of enrichment on locomotor habituation or hyperactivity in the open field, in contrast, can be produced either early (immediately postweaning) or late (adulthood, as in the current study). The effects of enrichment on Morris water maze performance are more complex in that the enrichment effects depend both on length of time in enrichment and age at exposure.

The timing of enrichment, therefore, appears to be a significant contributing factor in enrichment's effects of subsequent cognitive performance. Although enrichment has been found to slow cognitive decline in older rats or enhance performance of middle-aged rats, the greatest effects occur when enrichment is introduced early on in the organism's life. This effect is true of humans as well as of animals.

In this study, animals arrived at the facility at 43-45 days old and were placed in enrichment (or remained in isolation) at 54-55 days old. Older animals were used in this study to parallel the procedures in Experiment II. Previous studies using the fluid percussion procedure have generally used animals weighing between 250-400 grams, a size that maximizes animal survival following surgery. Future studies should include animals of different ages to determine whether age interacts with physical and social enrichment on the selected behavioral measures.

Experiment I: Summary and Implications

Environmental enrichment has well documented effects on brain development, brain function, and cognitive performance. These effects occur in both animals and humans. In animals, previous studies using rats have reported that environmental enrichment changes brain anatomy and neurochemistry and exerts beneficial effects on cognitive functioning (*e.g.,* Diamond 1967; van Praag *et al.,* 1999). These effects are most robust when enrichment is introduced early and for longer periods of time.

In humans, children reared in impoverished environments, typically characterized by lower socioeconomic conditions or homes void of emotional or parental support, exhibit impairments in cognitive and behavioral functioning (McEwen, 2000; Sanchez *et al*., 2001). Child intervention programs (*i.e.,* Head Start) that combine early environmental and social stimulation can offset these detriments in functioning (Ramey & Ramey, 1998). Further, children characterized as stimulation seekers, who create enriched environments for themselves, score higher on IQ tests and have superior scholastic and reading ability compared to non-stimulation seekers (Raine, Reynolds, Venables, & Mednick, 2002).

The current study replicates previous reports in the literature that enrichment enhances performance on a variety of cognitive measures. The current study extends previous reports in two important ways: 1) social enrichment is particularly valuable, and 2) males and females differ in their responses to enrichment effects.

To date, the term “environmental enrichment” has been loosely defined, but generally refers to the combination of physical and social stimulation. In previous studies, while it was clear that the isolated or impoverished organism, (*i.e*., human or animal) appeared to exhibit decrements in learning and memory, it was not clear whether these decrements were the result of the absence of physical stimulation or the lack of opportunities for social interaction. Results from previous studies have lead to the general conclusion that it is the combination of physical and social factors that are important for enhancing performance. The findings from the current study indicate that social enrichment is the key to maximizing cognitive performance.

Results of the current study also indicate that males and females differ in their sensitivity to physical and social aspects of the environment. The reason for these observed gender differences is not clear. Previous studies using animals and humans have indicated that males and females differ in cognitive abilities (Halpern, 1992; Linn & Petersen, 1985). These gender differences in cognitive ability may be explained by differences in brain architecture or hormonal fluctuations. In this experiment, the effects on enrichment on cognitive performance depended on animals gender and cognitive task. Specifically, social enrichment enhanced performance of males and females on a simple information-processing task (*i.e*., locomotor habituation). Physical enrichment enhanced performance on the same measure for males, but not for females. Social enrichment improved performance on a complex spatial memory (*i.e.,* Morris water maze), for males, but not for females. Physical enrichment improved sensory gating (*i.e*., %PPI) for females, but not for males.

It appears from these findings that gender differences in the effects of enrichment are task dependent. One potential explanation for these findings is that enrichment has differential effects on brain anatomy and neurochemistry. Only a few studies have examined this possibility and these studies have reported mixed results. Juraska and colleagues have reported sex differences in the effects of enrichment on the cortex of rats (*e.g.,* Juraska, 1984;1990). Specifically, male rats had larger changes in the visual cortex than did females and female rats had larger changes in the dentate gyrus of the hippocampal formation. Kolb and colleagues

(2003) recently reported that these gender-specific changes depend on animal age and are less likely to occur in adult animals.

The current results suggest that males and females may differ in the amount of exposure necessary to derive benefit from enrichment effects. In the study by Kolb (2003), animals were reared in enriched environments for 95 days. In the current study, testing was initiated at 11 days of enrichment (*e.g.,* locomotor habituation) and concluded at 35 days of enrichment (*e.g*., passive avoidance). Females may require a longer time in enrichment to derive benefits from enrichment effects. The fact that females exhibited the greatest effects on the last days of measurement is consistent with this possibility. Future studies should examine the response of females to enrichment following different periods of enrichment.

In addition to the differential gender effects found in this experiment and the possibility that timing of enrichment may account for these effects, another interesting finding emerged from the current experiment. The effects of enrichment were greatest on measures of locomotor habituation, passive avoidance, and Morris water maze. All of these tasks share a common element – the active processing of contextual information. In the locomotor chamber, processing of environmental cues is necessary to reduce exploration and increase habituation to a novel environment. In the passive avoidance procedure, animals must be able to make an association between a darkened chamber and the experience of shock. In the Morris water maze procedure, animals must utilize environmental cues to navigate their way through the maze. Enrichment, in contrast, had little effect on acoustic startle, a task that is independent of the active or volitional processing of information. Perhaps

enrichment facilitates learning and memory, primarily by increasing awareness and the active processing of relevant environmental cues and information.

The findings from Experiment I suggest that social interaction plays a key role to maximize cognitive performance, especially of more complex tasks. These findings may be relevant to humans in several ways. First, if these findings extend to humans, then early learning experiences should involve opportunities for social interaction, rather than individual instruction. While opportunities for physical stimulation (*i.e.*, video games, virtual reality) may play a contributory role, physical stimulation alone cannot produce the beneficial effects observed in this study. Further, because males and females differ in their response to enrichment, early learning experiences should be tailored to maximize learning for males and females. Specifically, males and females would benefit most from social enrichment (*e.g.,* learning in groups). Females, however, may require frequent and sustained social enrichment over time.

In addition to having implications for education and training, the finding that enrichment has the greatest effects to enhance the processing of contextual information may have important psychosocial consequences. That is, if enrichment affects efficient processing of contextual information, then environmental enrichment may prove to be an important tool in the management of psychological disorders such as anxiety and depression— conditions which have been linked to deficits in information processing. Alternatively, social enrichment may be a valuable tool to treat neurodevelopmental disorders characterized by deficits in processing novel information (*e.g.,* autism).

Table 8. Summary of Major Findings For Experiment II (Injured Animals)						
	Males Social	**Males Physical**	**Males Social X Physical**	**Females Social**	**Females Physical**	**Females Social X Physical**
Locomotion	**Improved on 17 & 28**	**Improved on ED 17**	No effect	**Improved on ED 12 & 17**	**Improved on ED 17**	No effect
ASR	No effect	No effect	No effect	No effect	No effect	No effect
% PPI -82	No effect	No effect	No effect	No effect	No effect	No effect
%-PPI -75	No effect	No effect	No effect	No effect	No effect	No effect
%-PPI-visual	No effect	No effect	No effect	No effect	No effect	No effect
PA Test	No effect	No effect	No effect	**Improved**	No effect	No effect
WM mean time	**Improved on ED 22**	**Improved on ED 24**	No effect	No effect	No effect	No effect
WM Trial I time	No effect	No effect	No effect	No effect	No effect	No effect
WM overall performance	**Improved**	**Improved**	*	**Improved**	**Improved**	*

*These results refer to the chi square analyses. Therefore, no interaction term is available.

Experiment II: Locomotor

As with Experiment I, locomotor activity was measured in injured animals to provide an index of simple information processing. Animals were placed in the activity chambers for 1 hour on ED 12, 17, and 28. When animals are first placed in the activity chambers, activity levels are high. As animals acclimate to the testing chamber, activity levels should decrease. Lower levels of activity or decreasing activity over time reflect more efficient information processing. In Experiment II, the

separate effects of enrichment (social vs. physical) on locomotor habituation depended on time in enrichment (*i.e.*, day of measurement) and animal gender.

For injured males, the effects of the physical environment appeared early and then disappeared, increasing habituation on ED 12 and to a lesser extent on ED 17. The pattern of effects for social enrichment differed from the effects observed in intact males. Specifically, the effects of social enrichment to increase habituation for injured males did not appear until ED 17. The effects of social enrichment to increase habituation for intact males were present on ED 12. Notably, when the effects of social did appear, the effect size for social enrichment was large, accounting for 30% of activity variance on ED 17 and 18% of activity variance on ED 27. These results suggest that social enrichment is the key to improve information processing in brain-injured males.

The effects of enrichment for brain-injured females, in contrast, followed a pattern more similar to intact males. Specifically, the effects of social enrichment appeared early (ED 12), persisted until enrichment day 17, and then disappeared. The effects of physical, which were present on enrichment day 17 for both injured males and females, were small compared to the effects of social enrichment and likely contributed little to the observed improvements in performance.

Overall, these results suggest that, like intact animals, brain-injured males and females vary in their response to enrichment. While males exhibit an early response to physical enrichment, they are most responsive to social aspects of the environment to which they respond more consistently over time. Females also appear more sensitive to the social aspects of the environment; however, these

effects do not persist over time and are not as large as the effects observed in males.

Only one other study has examined the effects of enrichment on locomotor activity in brain-injured animals (Farrell, Evans, & Corbett, 2001) and reported results consistent with the current findings. Farrell and colleagues examined the effects of enrichment on locomotor activity of female gerbils following ischemic cell death and reported that enriched (*i.e*., social and physical enrichment) animals exhibited lower activity and more rapid habituation to the testing chambers compared to isolated animals (Farrell *et al*., 2001). The effects of enrichment on locomotor habituation in brain-injured rats have not been examined previously.

Previous studies examining the effects of enrichment on brain injury recovery have focused on more complex cognitive tasks. The current study extends previous findings by demonstrating that: 1) enrichment enhances locomotor habituation in brain-injured rats; 2) social enrichment is the key factor responsible for these effects; and 3) brain-injured males are more responsive to the effects of social enrichment than are brain-injured females

Experiment II: ASR/PPI

As in Experiment I, the acoustic startle reflex was included in Experiment II to provide an additional measure of information processing. In Experiment II, enrichment had no effect to alter startle amplitude or pre-pulse inhibition. Only a few studies have examined the effects of brain injury on ASR amplitude. These studies have reported that brain-injury reduces startle amplitude. The current experiment

replicated those findings. To date, no studies have examined the effects of enrichment on startle amplitude or PPI in brain–injured animals. The current findings suggest that enrichment does not alter startle or PPI in brain-injured animals.

The finding that enrichment does not affect PPI in brain-injured animals does not preclude the possibility for enrichment to improve attention in the brain-injured animals or humans. Instead, it is possible that the effects of enrichment on attentional and information-processing tasks are complex and may depend on how the information is assimilated. Results from Experiment I suggested that enrichment affects tasks requiring the active processing of the environment, but has little effect on tasks in which processing occurs more passively (*i.e*., pre-consciously). In humans, passive processing might refer to the ability to filter out extraneous unrelated information, whereas active processing might refer to the ability to purposively direct ones attention toward a given aspect of the environment. Both types of processing can be characterized as aspects of attention; however, active processing involves a greater degree of intention on the part of the organism. If it were true that enrichment has the greatest effect to alter intentional behavior, then one would not expect to observe effects of enrichment on prepulse inhibition. The effect of enrichment on other aspects of attention, however, may be worth exploring in future studies with brain-injured animals.

Attentional deficits are common in brain-injured patients and can contribute to significant impairments in functioning. Deficits in sustained (*i.e.,* goal-directed attention), for example, interfere with the ability to recall previously learned

information or consolidate new information into memory. Further, deficits in attention may lead to misperceptions of the environment in ways that affect new learning or behavior. In order to further examine whether enrichment affects attentional processing in brain-injured animals, future studies should include more complex measures of attention. Only a few true animal paradigms of attention exist (Lawrence & Shakian, 1995). The five-choice SRRT task (Muier *et al*., 1995) – a measure of sustained attention in the rat or the contextual conditioning procedure -- a measure of attention to detail or discriminative ability (Woodcock & Richardson, 2000) may be more valuable ways to assess attention.

The five-choice serial reaction time test (SRTT) requires the rat to respond to discrete visual stimuli presented unpredictably in one of five spatial locations in order to obtain a food reward (Carli *et al*., 1983). To do well on the task, the rat must sustain attention over the entire task. This task also includes characteristics of divided attention (attending to all aspects of the environment) and visual search (Carli *et al*., 1983). This task has been used to assess the effects of forebrain cholinergic lesions (Muir *et al.,* 1995), drug actions (Turchi *et al*., 1995), and arousal (Hockey, 1984). The effects of environmental enrichment on this measure of attention have not been examined previously, but may be valuable to further understand the effect of enrichment to alter attention in ways that may affect high lever processing (*i.e*., water maze performance).

The contextual conditioning task may be valuable to assess attention to detail or discriminative abilities. The contextual conditioning task requires that the animal learns to discriminate between a conditioning context, where it was shocked, and a

similar context, where it was not shocked. Because this task requires more active processing of environmental cues, it may be more sensitive to enrichment effects.

Although the findings from ASR and PPI in Experiment II did not support the original hypothesis regarding enrichment effects, there are two important conclusions that can be drawn from the findings: 1) the effects of isolation housing on cognitive performance in brain-injured animals may be task dependent, and 2) PPI performance can be dissociated from isolation-induced hyperactivity. While the effects of enrichment to alter ASR or PPI appear dependent on a specific developmental period, the effects of enrichment on locomotor habituation or hyperactivity appear independent of developmental stage. Further, because isolation-induced hyperactivity is present in the absence of % PPI disruptions, hyperactivity cannot account for the increased reactivity that characterizes impairments in prepulse inhibition in brain-injured animals.

The absence of findings from ASR and PPI also does not support the hypothesis that performance in lower cognitive abilities (*i.e.*, PPI) would predict performance on more complex cognitive tasks. Attention is an essential component of new learning. Deficits in attention interfere with learning and may impair the consolidation of new information into memory. The finding that enrichment had no effect on PPI in brain-injured animals does not preclude the effect of enrichment to improve attention. Instead, it is possible that PPI does not provide the most valid measure of attention. Rather, PPI appears to be better characterized as an orienting response that prepares the organism to respond effectively to its environment. The animal is not actively learning anything new about its environment in a way that

would maximize learning or improve memory. Tasks such as the contextual conditioning task or the five-choice SRRT may provide a more accurate way of assessing the attentional abilities that underlie many of the complex cognitive processes that are compromised following TBI. Future studies should compare the effects of enrichment on other complex attentional tasks to determine whether this characterization is accurate.

Experiment II: Passive Avoidance

As with Experiment I, this simple memory task revealed the extent to which animals remembered that they had been shocked in a darkened chamber. All brain-injured animals were examined in the passive avoidance chamber. Enrichment had no effect on brain-injured males, but social enrichment improved performance of brain injured-females. Specifically, female brain-injured rats reared in social environments had significantly longer latencies to cross into the darkened chamber than did isolated animals. These results suggest that socially-enriched females had greater memory for the previous aversive event (*i.e.*, shock) than did isolation-reared animals.

The absence of findings in males did not appear to be the result of no enrichment effects. Instead, males generally performed well on this task. Among males there were no differences in the number of animals that crossed (did not remember) vs. did not cross (remembered), and this performance remained consistent regardless of enrichment status with enrichment neither improving nor impairing memory on this task. Among females, however, comparisons within

specific subgroups indicated that significantly more animals did not remember than did remember in each subgroup. The only exception was the SE (social only group) in which the number of animals that remembered equaled the number of animals that did not remember. These findings may suggest that the SE had some effects to improve memory.

The exact mechanism for the gender differences on this measure is less clear. Gender differences in passive avoidance performance following brain-injury or in response to enrichment have not been examined in the literature previously. However, sex differences in passive avoidance performance have been reported in the literature with females generally performing more poorly on the task than males. These reported gender differences in performance have been attributed, in part, to differences in pain sensitivity (Heinsbroek *et al*., 1988; Steenbergen *et al*., 1989, 1990; Van Oyen *et a*l., 1979). For females, pain sensitivity varies across the estrus cycle (Aloisi, 1997). It is not clear from the current results whether gender differences in performance can be attributed to learning or pain sensitivity. It is possible that enrichment's effect to improve performance on this measure may occur via alternations in pain sensitivity. Further studies could examine performance on this task at various shock intensities to determine how performance of males and females varies. If females do in fact demonstrate higher pain thresholds, then higher levels of shock may be necessary to obtain optimal results for females.

Experiment II: Morris water maze

As with intact animals, social enrichment had the greatest effect to improve performance on this task of spatial working memory and these effects were greatest in brain-injured males. In fact, there were no effects of enrichment on the performance of brain-injured females. Morris water maze was included in Experiment II to provide a more complex measure of cognitive performance (*i.e*., spatial learning and spatial memory). Further, the Morris water maze commonly is used to assess cognitive deficits associated with brain injury in rats. Previous studies utilizing this paradigm have reported that environmental enrichment enhances performance of brain-injured male rats. Only one study has compared the effects of environmental enrichment on the performance of male and female brain-injured rats. This study reported that environmental enrichment enhanced the spatial memory performance of male, but not female Sprague-Dawley rats (Wagner *et al*., 2002). The current results are consistent with Wagner *et al.'s* findings.

There are several possible explanations for the finding that brain-injured females are less responsive than are males to the effects of enrichment on water maze performance. It is possible, as Wagner and colleagues proposed, that males and females differ in their responses to enrichment, with females responding later than males. This explanation is consistent with the findings from the current study. Specifically, both intact and brain-injured females exhibited later responses to enrichment on other measures (*e.g*., locomotor habituation, water maze) when

compared to males. If this interpretation is correct, then females may require more prolonged exposure in enriched environments to obtain beneficial effects.

Another possibility is that hormonal fluctuations may affect both recovery from injury in females and the effects of enrichment on performance. Previous studies have reported that female sex hormones have a neuroprotective effect in females. The extent to which enrichment conditions interact with the hormones has not been examined previously but should be a focus of future studies.

It is also possible that the absence of findings in females may be attributable to strain differences. Specifically, previous studies examining the effects of enrichment in female rats have used Long-Evans females and reported that males and females do not differ in their response to enrichment. In the current experiment, male and female Sprague-Dawley rats differed in their responses to enrichment with female rats exhibiting less response to enrichment on most measures when compared to males. In the Wagner *et al*. study (2002), females also exhibited less response to enrichment when compared to males. Wagner and colleagues also used Sprague-Dawley rats. It is possible that the effects of enrichment in females depend on animal strain.

The gender differences observed in the current study also may reflect differences in performance baselines. That is, male rats generally perform better than females on the tasks used in this study (*i.e*., Morris water maze, passive avoidance). It is possible, therefore, that females have reached their maximum performance capacity and enrichment does little to improve their performance further. Including tasks on which females outperform males (*i.e*., radial arm maze or

social interaction) may be a better way to evaluate gender differences in response to enrichment.

In summary, then, the effects of enrichment in brain-injured animals followed a pattern similar to that reported in intact animals with social enrichment having the greatest effect on overall performance, and males exhibiting a greater response to enrichment than females. The effects of enrichment for males did not appear as robust as the effects reported in previous studies. Possible explanations for these weaker results include repeated testing and time in enrichment. This was only the second study that compared the responses of brain-injured males and females to environmental enrichment. The current finding that females are not as responsive to the effects of enrichment after brain injury replicates the previous findings (Wagner *et al*., 2002). The current findings do not make clear the reason for the weak findings in females, but strain or hormonal differences may be relevant.

As with Experiment I, there are several limitations of the current experiment that limit interpretation of the results.

Limitations of Experiment II

Some of the findings in Experiment II replicate reports in the literature and some findings did not. Factors that may have contributed to differences in observed effects include: 1) repeated behavioral testing, 2) length of time in enrichment, and 3) limited means of verifying injury. That is, injury was verified primarily by behavioral testing. It is possible that the animals varied in their neurological

response to the injury. Future studies should include tissue examination to complement cognitive and behavioral findings.

Repeated Behavioral Testing

As with Experiment I, it is possible that the lack of findings on some measures (*i.e*, Morris water maze) may be attributed, in part, to the fact that repeated tests were conducted on each animal over a relatively brief period of time. It is possible that repeated handling may have diminished effects of enrichment. Specifically, repeated testing on a wide variety of behavioral measures may have acted as a type of enrichment for the isolated animals. The methods used in Experiment II were selected to better understand how enrichment affects cognitive processing in brain-injured animals at different levels of complexity. Future designs should space out behavioral measures or include a control group that receives less handling to determine to what extent repeated behavioral testing might have similar effects as enrichment.

Timing of enrichment

In the current study, behavioral testing was started 11 days after injury and continued until 35 days post injury. While enrichment has been reported to exert beneficial effects even following brief periods of exposure, adult animals may require longer periods of enrichment to derive optimal benefit.

The timing of enrichment appears to be a significant factor in enrichment effects on subsequent cognitive performance. Although enrichment has been found to slow cognitive decline in older rats or enhance performance of middle-aged rats,

the most robust findings occur when enrichment is introduced early in the organism's life. This effect is true of humans as well as of animals. Animals reared in enriched conditions from several weeks post-weaning (*e.g*., 21 days) exhibit the greatest response to enrichment. In this study, animals were placed in enrichment at 54-55 days old. Older animals were used in this experiment to maximize survival from surgical procedures. That is, previous studies using the fluid percussion procedure have generally used animals weighing between 250-400 grams, a size consistent with the age of the animals when placed in enrichment. To date, no studies have examined the effects of enrichment at different time points following fluid percussion injury. Such a study might delineate the critical periods for enrichment effects following this type of injury.

Brain Injury Method

Another possible limitation of the current study and its potential implications for humans is the brain injury method used. The fluid percussion model is an impact-induced trauma model in which a column of fluid is propelled against the surface of the brain causing a focal injury at the point of impact as well as secondary damage from concomitant swelling and bleeding around the injured area. In humans, closed head injury typically involves injury at area of impact as well as axonal shearing due to rotational forces (*i.e*., movement of the head upon impact). Because the animal's head is stabilized during the fluid percussion injury, the potential for axonal shearing is minimal. Therefore, this model may not fully replicate the extent of damage commonly observed in brain-injured humans. Future studies should examine alternate models of brain injury (*e.g.,* cortical impact,

penetrating head injuries, stroke) to determine if the current results replicate across brain injury models.

Experiment II: Summary and Implications

Environmental enrichment has well-documented effects on brain development, brain function, and cognitive performance in intact animals (Diamond *et al*., 1994; Bennet *et al., 1996*). Recently, the effects of enrichment to improve cognitive recovery and decrease lesion volume have been reported in rats (Hamm *et a*l., 1996; Passineau, *et al*, 2001; Held, Gordon, & Gentile, 1985). The findings that enrichment may help to alleviate injury effects and improve recovery have been used to devise treatment for brain-injured humans (Rose, Davey, & Attree, 1993). The development of such treatments, however, has focused on the view that it is the social and physical aspects of enrichment together that are responsible for the observed treatment benefits. Therefore, treatment paradigms based on this literature have focused on developing ways that the brain-injured individual can interact with the environment to maximize motor and sensory stimulation.

The current research suggests that social enrichment is particularly important for post-injury rehabilitation. The addition of physical objects to the social environment does not appear to provide additional benefit on most measures of cognitive performance. If these findings extend to humans, then rehabilitation programs should focus on ways to incorporate opportunities for social interaction into the rehabilitation process. Examples of ways to achieve that goal may include opportunities for group physical and occupational therapy, interactive group therapy sessions, and an emphasis on social communication in speech therapy. This added

component of rehabilitation may further augment the recovery process and perhaps even alleviate the distress of the hospital environment.

OVERALL SUMMARY AND CONCLUSIONS

The goals of this doctoral research were to examine the separate effects of social enrichment (SE) and physical enrichment (PE) on cognitive performance of neurologically-intact and brain-injured rats and to determine if there were gender differences in these effects. Measures of basic and complex cognitive processing were included. Experiment I examined the effects of enrichment on performance of intact animals. Experiment II examined the effect of enrichment on performance of injured animals. The major findings from the current study were: 1) social enrichment has the greatest effect to improve performance for intact and injured animals; 2) the effects of social enrichment appear to be particularly important for males than for females; 3) enrichment has the greatest beneficial effect on tasks that require the active processing of information.

If these findings extend to humans, then they suggest that the social environment has important beneficial effects for cognitive performance and opportunities for social interaction should be considered when developing educational programs for intact individuals or rehabilitation programs for brain-injured individuals. The presence of physical objects within the social environment (PESE) does not appear to provide additional benefit on most measures of cognitive performance. Further, while males may derive immediate effects from enrichment,

females may require longer exposure to the social environment in order to obtain maximal results.

While the current results extend the previous literature in several ways, further studies are needed to help explain the gender differences in responding. That is, are females less responsive to enrichment or might the gender differences reported in this study be explained by strain or timing differences? Further studies would need to include rats of different strains as well as varying lengths of exposure to enrichment to answer these questions.

Another interesting question that emerged from these results is whether enrichment affects only those aspects of performance that involve the active processing of information. Future studies should include tasks that measure different types of information processing and attention to determine if the present findings extend to other measures of cognitive performance.

Finally, the current study focused only on the cognitive responses to enrichment. Future studies should examine the effects of enrichment on brain anatomy and neurochemistry to provide data that can complement the current results and provide explanations for the differential effects of social and physical enrichment. Particularly, it would be interesting to determine whether physical and social enrichment have different effects on the brain itself that may explain observed differences in performance and possibly the observed differences between the sexes.

REFERENCES

Acri, J.B. (1994). Nicotine modulates effects of stress on acoustic startle reflexes in rats: Dependence on dose, stressor, and initial reactivity. Psychopharmacology, 116, 255-265.

Aloisi, A.M. (1997). Sex differences in pain-induced effects on the septo-hippocampal system. Behavioral Brain Research 25, 397-405.

Altman, J., & Das, G.D. (1964). Autoradiographic examination of the effects of enriched environment on the rate of glial multiplication in the adult rat brain. Nature, 204, 1161-1163.

Bakshi, V.P., & Geyer, M.A. (1999). Ontongeny of isolation rearing-induced deficits in sensorimotor gating in rats. Physiology and Behavior, 67, 385-392.

Blair, R., Liran, J., Cytryniak, H., Shizgal, P., & Amit, Z. (1978). Explosive motor behavior, rigidity, and periaqueductal gray lesions. Neuropharmacology, 17, 205-209.

Bowling, S.L., Rowlett, J.K., & Bardo, M.T. (1993). The effect of environmental enrichment on amphetamine-stimulated locomotor activity, dopamine synthesis, and dopamine release. Neuropharmacology, 32, 885-893.

Braff, D.L., Swerdlow, N.R., & Geyer, M.A. (1999). Symptom correlates of prepulse inhibition deficits in male schizophrenic patients. American Journal of Psychiatry, 156, 596-602.

Brandeis, R., Brandys, Y., & Yehuda, S. (1989). The use of the Morris water maze in the study of memory and learning. International Journal of Neuroscience, 48, 29-69.

Brown, K.J., & Grunberg, N.E. (1995). Effects of housing on male and female rats: Crowding stresses males but calms females. Physiology and Behavior, 58, 1085-1089.

Brown, S.A., & Levin, H. S. (2001). Clinical presentation and neuropsychological sequelae of traumatic brain injury. In L. P. Miller & R. L. Hayes (Eds.). Head Trauma: Basic Preclinical and Clinical Directions. New York: John Wiley & Sons, 349-370.

Capruso, D.X. & Levin, H.S. (1992). Cognitive impairment following closed head injury. Neurologic Clinics, 10, 879-893.

Carli, M., Robbins, T.W., Evenden, J.L.,& Everitt, B.J. (1983). Effects of lesions to noradrendergic neurons on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. Behavioral Brain Research, 3, 361-380.

Center for Disease Control (2002). Traumatic brain injury among American Indians/Alaska Natives-United States, 1992-1996. Morbidity and Mortality Weekly Report, 51, 303-305.

Chapillon P., Manneche C., Belzung C., & Caston J. (1999). Rearing environmental enrichment in two inbred strains of mice: Effects on emotional reactivity. Behavioral Genetics, 1, 41-46.

Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences. Hillsdale, NJ: Lawrence Erlbaum Associates.

Coltheart, M., Hull, E., & Slater, D. (1975). Sex differences in imagery and reading. Nature, 253, 438-440.

Coover, G.D., & Levin, S. (1972). Auditory startle response of hippocampectomized rats. Physiology and Behavior 9, 75-77.

Crnic, L.S. (1983). Effects of nutrition and environment on brain biochemistry and behavior. Developmental Psychobiology, 16, 129-145.

Daniel, J.M., Roberts, S., & Dohanich, G. (1999). Effects of ovarian hormones and environment on radial maze and water maze performance of female rats. Physiology and Behavior, 66, 11-20.

Darwin, C. (1874). The Descent of Man (2nd ed.). London: John Murray.

Davis, M. (1984). The mammalian startle response. In: Eaton, R. (Ed.). Neural Mechanisms of Startle Behavior. New York: Plenum Press, 287-351.

De Courten-Meyers, G. M. (1999). The human cerebral cortex: Gender differences in structure and function. Journal of Neuropathology and Experimental Neurology, 58, 217-226.

Decker, M.W. (1995). Animal models of cognitive function. Critical Reviews of Neurobiology, 9, 321-343.

Diamond, M.C. (1967). Extensive cortical depth measurements and neuron size increases in the cortex of environmentally enriched rats. Journal of Comparative Neurology, 131, 357-364.

Diller, L. (1987). Neuropsychological rehabilitation. In M.J. Meier, A. Benton, & L. Diller (Eds.). Neuropsychological Rehabilitation. New York: Guilford Press, 3-17.

Dixon, C.E., Lyeth, B.G., Povlishock, J.T., Findling, R.L., Hamm, R.J., Marmarou, A., Young, H.F., & Hayes, R.L. (1987). A fluid percussion model of experimental brain injury in the rat. Journal of Neurosurgery, 67, 110-119.

Donders, J., & Woodward, H.R. (2003). Gender as a moderator of memory after traumatic brain injury in children. Journal of Head Trauma Rehabilitation, 18, 106-115.

Einon, D. (1980). Spatial memory and response strategies in rats: Age, sex, and rearing differences in performance. Quarterly Journal of Experimental Psychology, 32, 473-489.

Einon, D.F., & Morgan, M.J. (1977). A critical period for social isolation in the rat. Developmental Psychobiology, 10, 123-132.

Faraday, M.M., Rahman, M.A., Scheufele, P.M., & Grunberg, N.E. (1998). Nicotine impairs startle and sensory-gating in Long-Evans rats. Pharmacology Biochemistry and Behavior, 61, 281-289.

Faraday, M.M., O'Donoghue, V.A., & Grunberg, N.E. (1999a). Effects of nicotine and stress on startle amplitude and sensory gating depend on rat strain and sex. Pharmacology Biochemistry and Behavior, 62, 273-284.

Faraday, M.M., Scheufele, P.M., Rahman, M.A., & Grunberg, N.E. (1999b). Effects of chronic nicotine administration on locomotion depend on rat sex and housing condition. Nicotine and Tobacco Research, 1, 143-151.

Faraday, M.M., & Grunberg, N.E. (2000). The importance of acclimation in acoustic startle amplitude and pre-pulse inhibition testing in male and female rats. Pharmacology Biochemistry and Behavior, 66, 375-381.

Farid, M., Martinez, Z.A., Geyer, M.A., & Swerdlow, N.R. (2000). Regulation of sensorimotor gating of the startle reflex by serotonin 2A receptors. Ontogeny and strain differences. Neuropsychopharmacnology, 23, 623-632.

Gardner, E.B., Boitano, J.J., Mancino, N.S., & D'Amico, D.P. (1975). Environmental enrichment and deprivation: Effects on learning, memory and extinction. Physiology and Behavior, 14, 321-327.

Geyer, M.A., Swerdlow, N.R., Mansbach, R.S., & Braff, D.L. (1990). Startle response modes of sensorimotor gating and habituation deficits in schizophrenia. Brain Research Bulletin, 25, 485-498.

Geyer, M.A., Wilkinson, L.S., Humby, T., & Robbins, T.W. (1993). Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. Biological Psychiatry, 34, 361-372.

Gleason, T.C., Dreiling, J.L., & Crawley, J.N. (1999). Rat strain differences in response to galanin on the Morris water task. Neuropteptides, 33, 265-270.

Greenough, W.T. (1975). Experimental modification of the developing brain. American Scientist, 63, 37-46.

Greenough, W.T., Black, J.E., & Wallace, C.S. (1987). Experience and brain development. Child Development, 58, 539-559.

Gron, G., Wunderlich, A. P., Spitzer, M., Tomezak, R., & Riepe, M. (2000). Brain activation during human navigation: Gender different neural networks as substrate of performance. Nature Neuroscience, 3, 404-408.

Groswasser, Z., Cohen, M., & Keren, O. (1998). Female TBI patients recover better than males. Brain Injury, 12, 805-808.

Hamm, R.J., Lyeth, B.G., Jenkins, L.W., O'Dell, D.M., & Pike, B. (1993). Selective cognitive impairments following traumatic brain injury in rats. Behavioural Brain Research, 59, 169-173.

Hall, S., & Bornstein, R. A. (1991). The relationship between intelligence and memory following minor or mild closed head injury: Greater impairment of memory than intelligence. Journal of Neurosurgery, 75, 378-381.

Hall, F.S. (1998). Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioural consequences. Critical Review of Neurobiology, 12, 129-162.

Halpern, D.F. (1992). Sex differences in cognitive abilities (2nd ed.). Hillsdale, NJ: Erlbaum.

Hebb, D.O. (1947). The effects of early experience on problem solving at maturity. American Psychologist, 2, 307-308.

Heinsbroek, R.P., van Harren, F., Feenstra, M.G., Boon, P., & Van de Poll, N.E. (1991). Controllable and uncontrollable footshock and monaminergic activity in the frontal cortex of male and female rats. Brain Research, 551, 247-255.

Hickey, R.W., Akino, M., Strausbaugh, S., & De Courten-Meyers, G.M. (1996). Use of the Morris water maze and acoustic startle chamber to evaluate neurological injury after asphyxial arrest in rats. Pediatric Research, 39, 77-84.

Hockey, R. (1984). Varieties of attentional state: the effects of environment. In: Parasuraman R, Davies DR (eds). Varieties of Attention. Academic Press: Orlando, Fla., pp 449–483.

Hoffman, H.S., & Ison, J. R. (1980). Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. Psychological Review, 87, 175-189.

Hogg, S., Moser, P., & Sanger, D.J. (1998). Mild traumatic lesions of the right parietal cortex of the rat: Selective behavioural deficits in the absence of neurological impairment. Behavioural Brain Research, 93, 143-155.

Hooge, R.D., & De Deyn, P. (2001). Applications of the Morris water maze in the study of learning and memory. Brain Research Review, 36, 60-90.

Jenkins, L.W., Lu, Y.C., Johnston, W.E., Lyeth, B.G., & Prough, D.S. (1999). Combined therapy affects outcome differentially after mild traumatic brain injury and secondary forebrain ischemia in rats. Brain Research, 817, 132-144.

Jennett, B., Snoek, J., Bond, M.R., & Brooks, N. (1981). Disability after severe head injury: Observations on the use of the Glasgow Outcome Scale. Journal of Neurology Neurosurgery and Psychiatry, 44, 285-293.

Johansson, B.B., & Ohlsson, A.L. (1996). Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. Experimental Neurology, 129, 322-327.

Joseph, R. (1999). Environmental influences on neural plasticity, the limbic system, emotional development, and attachment: A review. Child Psychiatry and Human Development, 29, 189-208.

Juraska, J.M. (1984). Sex differences in dendritic responses to differential experience in the rat visual cortex. Brain Research, 295, 27-34.

Juraska, J.M., Fitch, J.M., Henderson, C., & Rivers, N. (1985). Sex differences in the dendritic branching of dentate granule cells following differential experiences. Brain Research, 333, 73-80.

Juraska J.M, & Kopcik J.R. (1988). Sex and environmental influences on the size and ultrastructure of the rat corpus callosum. Brain Research, 450, 1-8.

Juraska, J.M. (1990). The structure of the cerebral cortex: Effects of gender and the environment. In B. Kolb & R. Tees (Eds.). The Cerebral Cortex of the Rat (pp. 483-506). Cambridge, MA: MIT Press.

Kaler, S.R., & Freeman, B.J. (1994). Analysis of environmental deprivation: Cognitive and social development in Romanian orphans. Journal of Child Psychology and Psychiatry, 35, 768-781.

Katz, D. I., & Alexander, M.P. (1994). Traumatic brain injury. Predicting course of recovery and outcome for patients admitted to rehabilitation. Archives of Neurology, 51, 661-70.

Keppel, G. (1991). Design and Analysis: A Researcher's Handbook, (3rd edition). Upper Saddle River, NJ: Prentice Hall.

Keppel, G., Saufley, W., & Tokunaga, H. (1992). Introduction to Design & Analysis: A Student's Handbook (2nd ed), New York: W.H. Freeman and Company.

Kimura, D. (1992). Sex differences in the brain. Scientific American, 267, 118-125.

Kolb, B., & Gibb, R. (1991). Environmental enrichment and cortical injury: Behavioral and anatomical consequences of frontal cortex lesions in rats. Cerebral Cortex, 1, 189-198.

Kolb, B., Forgie, M., Gibb, R., Gorny, G., & Rowntree, S. (1998). Age, experience, and the changing brain. Neuroscience and Biobehavioral Reviews, 22, 143-159.

Kolb, B., Gibb R., & Gorny, G. (2003). Experience-dependent changes in dendritic arbor and spine density in neocortex vary qualitatively with age and sex. Neurobiology of Learning and Memory, 79, 1-10.

Kolb, B., & Whishaw I.Q. (1996). Fundamentals of human neuropsychology (4th edition). New York: W.H. Freeman.

Kotapka, M.J., Graham, D.I., Adams, J.H., & Gennarelli, T.A. (1994). Hippocampal pathology in fatal human head injury without high intracranial pressure. Journal of Neurotrauma, 11, 317-324.

Larsson, F., Winblad, B., & Mohammed, A.H. (2002). Psychological stress and environmental adaptation in enriched vs. impoverished housed rats. Pharmacology Biochemistry and Behavior, 73, 193-207.

Lauterbach, M., Raz, S., & Sander, C. (2001). Neonatal hypoxic risk in preterm birth infants: The influence of sex and severity of respiratory distress on cognitive recovery. Neuropsychology, 15, 411-420.

Lehmann, J., Pryce, C.R., & Feldon, J. (1999). Sex differences in the acoustic startle response and prepulse inhibition in Wistar rats. Behavioural Brain Research, 104, 113-117.

Ling, G.S., & Garcia-Pinto, P. (1999). Traumatic brain injury in the rat using the fluid percussion model. Current Protocols in Neuroscience, 9, 1-7.

Lu, J., Moochhala, S., Shirhan, M., Ng, K.C., Teo, A.L., Tan, M.H., Moore, X.L., Wong, M.C., Ling, E.A. (2003). Neuroprotection by aminoguanidine after lateral fluid-percussive brain injury in rats: a combined magnetic resonance imaging, histopathologic and functional study. Neuropharmacology, 44, 253-63.

Maccoby, E.E., & Jacklin, C.N. (1978). The Psychology of Sex Differences. Stanford, CA: Stanford University Press.

Matiasson, G.J., Philips, M.F., Tomasevic, G., Johansson, B.B., Wieloch, T., & McIntosh, T.K. (2000). The rotating pole test: evaluation of its effectiveness in assessing functional motor deficits following experimental head injury in the rat. Journal of Neuroscience Methods, 95, 75-82.

McEwen, B.S. (2000). Effects of adverse experiences for brain structure and function. Biological Psychiatry, 48, 721-731.

McIntosh, T.K., Vink, R., Noble, L., Yamakami, I., Fernyak, S., Soares, H., & Faden, A.L. (1989). Traumatic brain injury in the rat: Characterization of a lateral fluid-percussion model. Neuroscience, 28, 233-344.

Mittl, R.L., Grossman, R.I., Hiehle, J.F., Hurst, R.W., Kauder, D.R., Gennarelli, T.A., & Alburger, G.W. (1994). Prevalence of MR evidence of diffuse axonal injury in patients with mild head injury and normal head CT findings. American Journal of Neuroradiology, 21, 808-809.

Muir, J.L., Everitt, B.J., & Robbins, T.W. (1995). Reversal of visual attentional dysfunction following lesions of the cholinergic basal forebrain by physostigmine and nicotine but not by the 5-HT3 receptor antagonist, ondanestron. Psychopharmacology, 118, 82-92.

National Institutes of Health (1999). Consensus conference. Rehabilitation of persons with traumatic brain injury. NIH Consensus Development Panel on Rehabilitation of Persons With Traumatic Brain Injury. Journal of the American Medical Association, 282, 974-83.

Nieman, H., Ruff, R.M., & Kramer, J.H. (1996). An attempt towards differentiating attentional deficits in traumatic brain injury. Neuropsychological Review, 6, 11-46.

Ohlsson, A.L., & Johansson, B.B. (1995). Environmental influences functional outcome of cerebral infarction in rats. Stroke, 26, 644-649.

Passineau, M.J., Green, E.J., & Dietrich, D. (2001). Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats. Experimental Neurology, 168, 373-384.

Paulus, M.P., Bakshi, V.P., & Geyer, M.A. (1998). Isolation rearing affects sequential organization of motor behavior in post-pubertal Lister and Sprague-Dawley rats. Behavioural Brain Research, 94, 271-280.

Petit, T.L, & Alfano, D.P. (1979). Differential experience following developmental lead exposure: effects on brain and behavior. Pharmacology Biochemistry and Behavior, 11, 165-171.

Pham, T.M., Ickes, B., Albeck, D., Soderstrom, G., & Mohammed, A.H. (1999). Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. Neuroscience, 94, 279-286.

Raine, A., Reynolds, C., Venables, P.H., & Mednick, S.A. (2002). Stimulation seeking and intelligences: a prospective longitudinal study. Journal of Personality and Social Psychology, 82, 663-674.

Ramey, C.T., & Ramey, S.L. (1998). Early intervention and early experience. American Psychologist, 53, 109-120.

Raz, S., Goldstein, R., Hopkins, T., & Lauterbach, M.D. (1994). Sex differences in early vulnerability to cerebral injury and their neurodevelopmental implications. Psychobiology, 22, 244-253.

Robbins, T.W., Jones, G.H., & Wilkinson, L.S. (1996). Behavioural and neurochemical effects of early social deprivation in the rat. Journal of Psychopharmacology, 10, 39-47.

Roof, R.L., & Hall, E. D. (2000). Estrogen-related gender difference in survival rate and cortical blood flow after impact-acceleration head injury in rats. Journal of Neurotrauma, 17, 1155-1169.

Rosenzweig, M.R. (1966). Environmental complexity, cerebral change, and behavior. American Psychologist, 21, 321-332.

Rosenzweig, M.R., Krech, D., Bennett, E.L., & Diamond, M. (1962). Effects of environmental complexity and training on brain chemistry and anatomy. A replication and extension. Journal of Comparative and Physiological Psychology, 55, 429-437.

Rosenzweig, M.R., & Bennett, E.L. (1996). Psychobiology of plasticity: Effects of training and experience on brain and behavior. Behavioural Brain Research, 78, 57-65.

Rosenzweig, M.R., Bennett, E.L., & Diamond, M.C. (1972). Brain changes in response to experience. Scientific American, 226, 22-29.

Sanchez, M.M., Ladd, C.O., & Plotsky, P.M. (2001). Early adverse experience as a developmental risk factor for later psychopathology: Evidence from rodent and primate models. Developmental Psychopathology, 13, 419-449.

Sanders, M.J., Dietrich, W.D., & Green, E.J. (1999). Cognitive function following traumatic brain injury: Effects of injury severity and recovery period in a parasagittal fluid-percussive injury model. Journal of Neurotrauma, 17, 915-925.

Schrijver, N.C., Bahr, N.I., Weiss, I.C., & Wurbel, H. (2002). Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. Pharmacology Biochemistry and Behavior, 73, 209-224.

Seymore, P., Dou, H., & Juraska, J.M. (1996). Sex differences in radial arm maze performance: Influence of rearing environment and room cues. Psychobiology, 24, 33-36.

Simpson, J.A. (1989). The Oxford English Dictionary. Oxford, England: Oxford University Press, Incorporated.

Skelton, R.W. (1998). Modeling recovery of cognitive function after traumatic brain injury: spatial navigation in the Morris water maze after complete or partial transactions of the perforant path in rats. Behavioural Brain Research, 96, 13-35.

Smith, H.V. (1972). Effects of environmental enrichment on open-field activity and Hebb-Williams problem solving in rats. Journal of Comparative and Physiological Psychology, 80, 163-168.

Speck, O., Earnst, T., Braun, J., Koch, C., Miller, E., & Chang, L. (2000). Gender differences in the functional organization of the brain for working memory. Neuroreport, 11, 2581-2585.

Spikman, J.M., Deelman, B.G., & van Zomeren, A.H. (2000). Executive functioning, attention, and frontal lesions in patients with chronic CHI. Journal of Clinical and Experimental Neuropyschology, 22, 325-338.

Springer, S., & Deutsch, G. (1998). Left Brain, Right Brain. New York: WH Freeman.

Stein, D. (2001). Brain damage, sex hormones and recovery: a new role for progesterone and estrogen? Trends in Neuroscience, 24, 386-391.

Swerdlow, N., Braff, D., & Geyer, M. (2001). Animal models of deficient sensorimotor gating: What we know, what we think we know, and what we hope to know soon. Behavioral Pharmacology, 11, 185-204.

Taylor, H. G., Yeates, K. O.**,** Wade, S. L., Drotar, D., Stancin, T., & Minich, M. (2002). A prospective study of short- and long-term outcomes after traumatic brain injury in children: Behavior and achievement. Neuropsychology, 16, 15-27.

Turchi, J., Holley, L.A., & Sarter, M. (1995) Effects of nicotinic acetylcholine receptor ligands on behavioral vigilance in rats. Psychopharmacology 118: 195–205

Van der Staay, F.J., & Blokland, A. (1996). Behavioral differences between outbred Wistar, inbred Fischer 344, brown Norway and hybrid Fischer 344 x brown Norway rats. Physiology and Behavior, 60, 97-109.

Van Praag, H., Kempermann, G., & Gage, F.H. (1999). Running increases cell proliferation and neuorgenesis in the adult mouse dentate gyrus. Nature Neuroscience, 2, 266-270.

Van Waas, M., & Soffie, M. (1996). Differential environmental modulations on locomotor activity, exploration and spatial behavior in young and old rats. Physiology and Behavior, 59, 265-271.

Van Zomeren, A.H., & Brouwer, W.H. (1994). Closed head injury. Clinical Neuropsychology of Attention. New York: Oxford University Press, 63-88.

Varty, G.B., Paulus, M.P., Braff, D.L., & Geyer, M.A. (2000). Environmental enrichment and isolation rearing in the effects on locomotor behavior and startle response . Biological Psychiatry, 47, 864-873.

Wagner A.K., Kline A.E., Sokoloski J., Zafonte R.D., Capulong E, & Dixon C.E. (2002). Intervention with environmental enrichment after experimental brain trauma enhances cognitive recovery in male but not female rats. Neuroscience Letter, 334; 165-168.

Wiley, J.L., Compton, A.D., Pike, B.R., Temple, M.D., McElderry, J.W., & Hamm, R.J. (1996). Reduced sensorimotor reactivity following traumatic brain injury in rats. Brain Research, 716, 47-52.

Wilkinson, L.S., Killcross, S.S., Humby, T., Hall, F.S., Geyer, M.A., & Robbins, T.W. (1994). Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. Neuropsychopharmacology, 10, 61-72.

Williams, B.M., Luo, Y., Ward, C., Redd, K., Gibson, R., Kuczaj, S.A., & McCoy, J. G. (2001). Environmental enrichment: Effects on spatial memory and hippocampal CREB immunoreactivity. Physiology & Behavior, 73, 649-658.

Wiley, J.L., Compton, A.D., Pike, B.R., Temple, M.D., McElderry, J.W., & Hamm, R.J. (1996). Reduced sensorimotor reactivity following traumatic brain injury in rats. Brain Research,716, 47-52,

Woodcock, E.A., & Richardson, R. (2000). Effects of environment enrichment on rate of contextual processing and discriminative ability in adult rats. Neurobiology of Learning and Memory, 73, 1-10.

Yeates, K.O., Taylor, H.G., Drotar, D., Wade, S.L., Klein, S., Stancin, T., & Schatschneider, C. (1997). Preinjury family environment as a determinant of recovery from traumatic brain injuries in school-age children. Journal of International Neuropsychological Society, 3, 617-630.

Zimmerman, A., Stauffacher, M., Langhans, W., & Wurbel, H. (2001). Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. Behavioural Brain Research, 121, 11-20.

APPENDIX A: TABLES FOR EXPERIMENT I

Table 9. Intact Animals: Results of repeated-measures ANOVAS on horizontal activity (baseline to ED 28)			
Experimental Group	**Effect**	**F value (d.f.)**	**p value**
All Animals	Time	0.117 (3, 486)	p = 0.950
	Time X Gender	2.428 (3, 486)	p = 0.065
	Time X Social	2.961 (3, 486)	p <0.05
	Time X Physical	0.853 (3, 486)	p = 0.466
	Time X Gender X Social	0.509 (3, 486)	p =0.676
	Time X Gender X Physical	1.662 (3, 486)	p = 0.174
	Time X Social X Physical	0.710 (3, 486)	p = 0.546
	Time X Gender X Social X Physical	0.097 (3, 486)	p = 0.961
Males	Time	0.909 (3, 216)	p = 0.437
	Time X Social	2.682 (3, 216)	p = 0.048
	Time X Physical	1.845 (3, 216)	p= 0.140
	Time X Social X Physical	0.802 (3, 216)	p = 0.494
Females	Time	1.674 (3, 270)	p =0.173
	Time X Social	1.179 (3, 270)	p = 0.318
	Time X Physical	0.931 (3, 270)	p = 0.426
	Time X Social X Physical	0.138 (3, 270)	p = 0.937

Table 10. Intact Animals: Results of univariate ANOVAS on horizontal activity averaged across baseline to ED 28			
Experimental Group	**Effect**	**F value (d.f.)**	**p value**
All Animals	Gender	23.073 (1, 162)	p < 0.001
	Social	17.119 (1, 162)	p < 0.001
	Physical	0.264 (1, 162)	p = 0.608
	Social X Physical	0.222 (1, 162)	p = 0.638
	Gender X Social	1.301 (1, 162)	p = 0.256
	Gender X Physical	0.000 (1, 162)	p =0.989
	Gender X Social X Physical	2.472 (1, 162)	p = 0.118
Males[1]	Social	10.500 (1, 72)	p < 0.05
	Physical	1.354 (1, 72)	p = 0.249
	Social X Physical	1.244 (1, 72)	p =0.268
Females	Social	7.054 (1, 90)	p < 0.05
	Physical	0.206 (1, 90)	p = 0.651
	Social X Physical	1.274 (1, 90)	p =0.262

[1] Note that the sample size (n) for males and females differs because several animals were excluded from the analyses due to equipment malfunction (see results section)

Table 11. Intact Animals: Results of ANOVAS on baseline activity			
Group Tested	**Dependent Variable**	**Univariate F value (d.f.)**	**p value**
All Animals	Gender	20.460 (1, 184)	p < 0.001
	Group	0.050 (3, 184)	p = 0.985
	Gender X Group	0.521 (3, 184)	p = 0.668
Males	Group	0.239 (3, 92)	p = 0.860
Females	Group	0.327 (3, 92)	p = 0.806

Table 12. Intact Animals: Results of univariate ANOVAS on horizontal activity for each measurement day when all animals were considered together ED 12-ED 28			
Day	**Effect**	**F value (d.f.)**	**p value**
ED (12)	Gender	21.698 (1, 182)	p <0.001
	Social	26.857 (1, 182)	p <0.001
	Physical	0.058 (1, 182)	p = 0.810
	Gender X Social	0.168 (1, 182)	p = 0.683
	Gender X Physical	7.422 (1, 182)	p < 0.05
	Social X Physical	0.451 (1, 182)	p = 0.503
	Gender X Social X Physical	1.796 (1, 182)	p = 0.182
ED (17)	Gender	0.908 (1, 168)	p = 0.342
	Social	12.463 (1, 168)	p <0.001
	Physical	1.715(1, 168)	p = 0.192
	Gender X Social	1.858 (1, 168)	p = 0.175
	Gender X Physical	0.140(1, 168)	p = 0.182
	Social X Physical	0.847(1, 168)	p = 0.359
	Gender X Social X Physical	0.606(1, 168)	p <0.001
ED (28)	Gender	18.965 (1, 180)	p <0.001
	Social	8.592 (1, 180)	p <0.05
	Physical	0.012 (1, 180)	p = 0.913
	Gender X Social	0.830 (1, 180)	p = 0.364
	Social X Physical	0.272 (1, 180)	p = 0.603
	Gender X Social X Physical	0.015 (1, 180)	p = 0.904
	Gender X Social X Physical	0.985 (1, 180)	p = 0.322

Table 13. Intact Males: Results of univariate ANOVAs on horizontal activity analyzed on each measurement day ED 12-ED 28			
Males	**Effect**	**F value (d.f.)**	**p value**
ED 12	Social	19.56 (1, 92)	p <0.001
	Physical	5.50 (1, 92)	p <0.05
	Social X Physical	2.53 (1, 92)	p = 0.115
ED 17	Social	12.971 (1, 76)	p <0.001
	Physical	1.535 (1, 76)	p = 0.219
	Social X Physical	0.011 (1, 76)	p = 0.917
ED 28	Social	3.077 (1, 88)	p = 0.083
	Physical	0.128 (1, 88)	p = 0.721
	Social X Physical	0.573 (1, 88)	p = 0.451

Table 14. Intact Females: Results of univariate ANOVAs on horizontal activity analyzed on each measurement day ED 12-ED 28			
Females	**Effect**	**F value (d.f.)**	**p value**
ED 12	Social	9.419 (1, 90)	p <0.05
	Physical	2.549 (1, 90)	p = 0.114
	Social X Physical	0.185 (1, 90)	p = 0.668
ED 17	Social	2.293 (1, 92)	p = 0.133
	Physical	0.428 (1, 92)	p = 0.515
	Social X Physical	1.410 (1, 92)	p = 0.238
ED 28	Social	5.651 (1, 92)	p <0.05
	Physical	0.152 (1, 92)	p = 0.697
	Social X Physical	0.474 (1, 92)	p = 0.493

Table 15. Intact Animals: Results of repeated-measures ANOVAs on horizontal activity within session (ED 12)			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
All Animals	Time	347.94 (11, 2002)	p <0.001
	Time X Gender	1.83 (11, 2002)	p < 0.05
	Time X Social	4.95 (11, 2002)	p <0.001
	Time X Physical	1.31 (11, 2002)	p = 0.211
	Time X Gender X Social	1.63 (11, 2002)	p =0.085
	Time X Gender X Physical	0.57 (11, 2002)	p =0.852
	Time X Social X Physical	0.47 (11, 2002)	p =0.921
	Time X Gender X Social X	1.91 (11, 2002)	p < 0.05
Males	Time	189.29 (11, 1012)	p <0.001
	Time X Social	4.01 (11, 1012)	p <0.001
	Time X Physical	0.84 (11, 1012)	p =0.596
	Time X Social X Physical	1.43 (11, 1012)	p =0.155
Females	Time	163.07 (11, 990)	p <0.001
	Time X Social	2.73 (11, 990)	p < 0.050
	Time X Physical	1.01 (11, 990)	p =0.431
	Time X Social X Physical	1.01 (11, 990)	p =0.438

Table 16. Intact Animals: Results of repeated-measures ANOVAs on horizontal activity within session (ED 17)			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
All Animals	Time	325.71 (11, 1848)	p <0.001
	Time X Gender	2.76 (11, 1848)	p <0.001
	Time X Social	3.73 (11, 1848)	p <0.001
	Time X Physical	0.69 (11, 1848)	p = 0.746
	Time X Gender X Social	1.12 (11, 1848)	p = 0.344
	Time X Gender X Physical	0. 93 (11, 1848)	p = 0.509
	Time X Social X Physical	1.12 (11, 1848)	p = 0.341
	Time X Gender X Social X	0.60 (11, 1848)	p = 0.830
Males	Time	139.98 (11, 836)	p <0.001
	Time X Social	2.93 (11, 836)	p <0.001
	Time X Physical	0.81 (11, 836)	p = 0.636
	Time X Social X Physical	1.20 (11, 836)	p = 0.283
Females	Time	194.05 (11, 1012)	p <0.001
	Time X Social	1.96 (11, 1012)	p <0.05
	Time X Physical	0.84 (11, 1012)	p = 0.597
	Time X Social X Physical	0.53 (11, 1012)	p = 0.885

Table 17. Intact Animals: Results of repeated-measures ANOVAs on locomotor activity within session (ED 28)

Treatment Group	Effect	F value (d.f.)	p value
All Animals	Time	275.33 (11, 1980)	p <0.001
	Time X Gender	3.15 (11, 1980)	p <0.001
	Time X Social	1.62 (11, 1980)	p =0.087
	Time X Physical	1.91 (11, 1980)	p <0.05
	Time X Gender X Social	0.90 (11, 1980)	p =0.542
	Time X Gender X	1.20 (11, 1980)	p =0.279
	Time X Social X	0.59 (11, 1980)	p =0.836
	Time X Gender X Social	1.01 (11, 1980)	p =0.436
Males	Time	165.71 (11, 968)	p <0.001
	Time X Social	0.40 (11, 968)	p =0.958
	Time X Physical	1.46 (11, 968)	p =0.140
	Time X Social X	1.35 (11, 968)	p =0.192
Females	Time	114.99 (11, 1012)	p <0.001
	Time X Social	2.08 (11, 1012)	p <0.05
	Time X Physical	1.65 (11, 1012)	p =0.079
	Time X Social X	0.29 (11, 1012)	p =0.988

Table 18. Intact Animals: Results of univariate ANOVAS on horizontal activity within session averaged across 1-hour testing session on ED 12

Treatment Group	Effect	F value (d.f.)	p value
All Animals	Gender	21.16 (1, 182)	p < 0.001
	Social	26.61 (1, 182)	p < 0.001
	Physical	0.10 (1, 182)	p =0.747
	Gender X Social	0.16 (1, 182)	p =0.689
	Gender X Physical	6.97 (1, 182)	p <0.05
	Social X Physical	0.34 (1, 182)	p =0.563
	Gender X Social X Physical	1.90 (1, 182)	p =0.170
Males	Social	18.57 (1, 92)	p < 0.001
	Physical	5.28 (1, 92)	p <0.05
	Social X Physical	2.304 (1, 92)	p =0.132
Females	Social	9.63 (1, 90)	p <0.05
	Physical	2.28 (1, 90)	p =0.134
	Social X Physical	0.27 (1, 90)	p =0.604

Table 19. Intact Animals: univariate ANOVAs on horizontal activity within session averaged across 1-hour testing session on ED 17

Treatment Group	Effect	F value (d.f.)	p value
All Animals	Gender	0.91 (1, 168)	p =0.342
	Social	12.46 (1, 168)	p < 0.001
	Physical	1.72 (1, 168)	p =0.192
	Gender X Social	1.86 (1, 168)	p =0.175
	Gender X Physical	0.14 (1, 168)	p =0.709
	Social X Physical	0.85 (1, 168)	p =0.359
	Gender X Social X Physical	0.67 (1, 168)	p =0.437
Males	Social	12.97 (1, 76)	p < 0.001
	Physical	1.54 (1, 76)	p =0.219
	Social X Physical	0.011 (1, 76)	p =0.917
Females	Social	2.29 (1, 92)	p =0.133
	Physical	0.43 (1, 92)	p =0.515
	Social X Physical	1.41 (1, 92)	p =0.238

Table 20. Intact Animals: Results of univariate ANOVAs on horizontal activity within session averaged across 1-hour testing session on ED 28

Treatment Group	Effect	F value (d.f.)	p value
All Animals	Gender	18.99 (1, 180)	p <0.001
	Social	8.93 (1, 180)	p <0.05
	Physical	0.003 (1, 180)	p =0.985
		0.73 (1, 180)	p =0.394
	Gender X Physical	0.33 (1, 180)	p =0.564
	Social X Physical	0.02 (1, 180)	p =0.903
	Gender X Social X Physical	0.98 (1, 180)	p =0.323
Males	Social	3.44 (1, 88)	p =0.067
	Physical	0.21 (1, 88)	p =0.650
	Social X Physical	0.57 (1, 88)	p =0.453
Females	Social	5.65 (1, 92)	p <0.05
	Physical	0.15 (1, 92)	p =0.697
	Social X Physical	0.47 (1, 92)	p =0.493

Table 21. Intact Animals: Results of MANOVAs on baseline startle amplitudes and PPI values				
All Animals	**Multivariate Effect and F value (d.f.)**	**Dependent Measure**	**Univariate F value (d.f.)**	**p value**
All animals	Gender 4.400 (4, 181) p = 0.002	Startle to 120 db	6.432 (1, 184)	p <0.05
		82 db pp	1.411 (1, 184)	p =0.236
		75 db pp	4.110 (1, 184)	p <0.05
		Visual pp	1.685 (1, 184)	p = 0.196
	Group 1.560 (12, 549) p =0.099	Startle to 120 db	1.165 (3, 184)	p =0.325
		82 db pp	1.280 (3, 184)	p =0.282
		75 db pp	2.884 (3, 184)	p <0.05
		Visual pp	2.865 (3, 184)	p <0.05
	Gender X Group 1.977 (12, 549) p =0.024	Startle to 120 db	1.880 (3, 184)	p =0.135
		82 db pp	0.328 (3, 184)	p =0.805
		75 db pp	3.704 (3, 184)	p <0.05
		Visual pp	1.466(3, 184)	p=0.225
Males	Group 0.488 (12, 273) p =0.921	Startle to 120 db	0.257 (3, 92)	p=0.856
		82 db pp	0.391(3, 92)	p=0.759
		75 db pp	0.039(3, 92)	p =0.990
		Visual pp	0.300(3, 92)	p=0.825
Females	Group 2.639 (12, 273) p =.002	Startle to 120 db	2.407 (3, 92)	p =0.072
		82 db pp	1.128(3, 92)	p=0.342
		75 db pp	5.575(3, 92)	p <0.05
		Visual pp	3.380(3, 92)	p <0.05

Table 22. Intact Animals: Results of repeated-measures ANOVAs on Startle Amplitude (baseline- ED 30)			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
Males	Time	124.11 (3, 276)	p <0.001
	Time X Social	0.33 (3, 276)	p =0.803
	Time X Physical	0.74 (3, 276)	p =0.526
	Time X Social X Physical	2.06 (3, 276)	p =0.106
Females	Time	30.11 (3, 276)	p <0.001
	Time X Social	3.62 (3, 276)	p <0.05
	Time X Physical	1.28 (3, 276)	p =0.281
	Time X Social X Physical	1.21 (3, 276)	p =0.307

Table 23. Intact Animals: Results of univariate ANOVAs on startle amplitude averaged across baseline to ED 30			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
Males	Social	0.01 (1, 92)	p =0.912
	Physical	1.46 (1, 92)	p =0.229
	Social x Physical	0.40 (1, 92)	p =0.529
Females	Social	1.64 6(1, 92)	p =0.203
	Physical	2.77 (1, 92)	p =0.100
	Social x Physical	0.79 (1, 92)	p =0.377

Table 24. Results of repeated-measures ANOVAs on % PPI-82 (ED 15- ED 30): baseline covaried			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
Males	Time	0.25 (2, 182)	p = 0.788
	Time X Baseline	0.23(2, 182)	p = 0.795
	Time X Social	0.21(2, 182)	p = 0.810
	Time X Physical	0.86 (2, 182)	p = 0.426
	Time X Social X Physical	1.86 (2, 182)	p = 0.159
Females	Time	6.55 (2, 182)	p <0.05
	Time X Baseline	3.88 (2, 182)	p <0.05
	Time X Social	1.48 (2, 182)	p = 0.231
	Time X Physical	3.07 (2, 182)	p <0.050
	Time X Social X Physical	2.22 (2, 182)	p <0.111

Table 25. Intact Animals: Results of Univariate ANOVAs % PPI-82 dB averaged across days (baseline covaried)			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
Males	Baseline	15.60 (1, 91)	p <0.001
	Social	3.50 (1, 91)	p= 0.065
	Physical	0.14 (1, 91)	p= 0.712
	Social x Physical	4.94 (1, 91)	p <0.050
Females	Baseline	18.67 (1, 91)	p <0.001
	Social	0.08 (1, 91)	p= 0.772
	Physical	0.51 (1, 91)	p= 0.477
	Social x Physical	4.47 (1, 91)	p <0.050

Table 26. Intact Animals: Results of repeated-measures ANOVAs on % PPI-75 (ED 15- ED 30): baseline covaried			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
Males	Time	0.37 (2, 182)	p =0.690
	Time X Baseline	0.42 (2, 182)	p =0.658
	Time X Social	0.44 (2, 182)	p =0.646
	Time X Physical	0.17 (2, 182)	p =0.844
	Time X Social X Physical	2.50 (2, 182)	p =0.085
Females	Time	4.63 (2, 182)	p <0.050
	Time X Baseline	1.84 (2, 182)	p =0.016
	Time X Social	3.07 (2, 182)	p <0.050
	Time X Physical	2.44 (2, 182)	p =0.090
	Time X Social X Physical	3.20 (2, 182)	p <0.050

Table 27. Intact Animals: Results of univariate ANOVAs % PPI-75 dB averaged across ED 15-30			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
Males	Baseline	12.19 (1, 91)	p <0.001
	Social	5.19 (1, 91)	p <0.050
	Physical	0.003 (1, 91)	p =0.960
	Social x Physical	10.38 (1, 91)	p <0.050
Females	Baseline	14.37 (1, 91)	p <0.001
	Social	0.53 (1, 91)	p =0.469
	Physical	5.47 (1, 91)	p <0.050
	Social x Physical	0.34 (1, 91)	p =0.563

Table 28. Intact Animals: Results of repeated-measures ANOVAs on % PPI-visual (ED 15-ED 30): baseline covaried			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
Males	Time	2.40 (2, 182)	p =0.093
	Time X Baseline	0.28 (2, 182)	p =0.754
	Time X Social	0.39 (2, 182)	p =0.678
	Time X Physical	1.94 (2, 182)	p =0.146
	Time X Social X Physical	2.40 (2, 182)	p =0.093
Females	Time	8.29 (2, 182)	p<0.001
	Time X Baseline	4.66 (2, 182)	p <0.050
	Time X Social	0.67 (2, 182)	p =0.516
	Time X Physical	1.46 (2, 182)	p =0.236
	Time X Social X Physical	0.004 (2, 182)	p =0.996

Table 29. Intact Animals: Results of univariate ANOVAs % PPI-visual averaged across days			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
Males	Baseline	28.34 (1, 91)	p <0.001
	Social	1.79 (1, 91)	p =0.185
	Physical	6.31 (1, 91)	p <0.050
	Social x Physical	1.16 (1, 91)	p =0.284
Females	Baseline	24.34 (1, 91)	p <0.001
	Social	0.57 (1, 91)	p =0.454
	Physical	0.18 (1, 91)	p =0.671
	Social x Physical	3.53 (1, 91)	p =0.063

Table 30. Intact Animals: Results of Univariate ANOVAs % PPI-82 dB on each enrichment day			
Males	**Effect**	**F value (d.f.)**	**p value**
ED 15	Social	4.6 (1, 91)	p <0.050
	Physical	0.15 (1, 91)	p =0.697
	Social x Physical	6.92 (1, 91)	p <0.05
ED 20	Social	1.59 (1, 91)	p =0.211
	Physical	1.01 (1, 91)	p =0.318
	Social x Physical	5.03 (1, 91)	p <0.050
ED 30	Social	1.16 (1, 19)	p =0.285
	Physical	0.01 (1, 91)	p =0.916
	Social x Physical	0.22 (1, 91)	p =0.643
Females	Effect	F value (d.f.)	p value
ED 15	Social	0.17 (1, 91)	p =0.685
	Physical	0.005 (1, 91)	p =0.943
	Social x Physical	3.5 (1, 91)	p =0.065
ED 20	Social	1.23 (1, 91)	p =0.271
	Physical	0.63 (1, 91)	p =0.431
	Social x Physical	0.04 (1, 91)	p =0.837
ED 30	Social	2.37 (1, 91)	p =0.127
	Physical	11.16 (1, 91)	p <0.001
	Social x Physical	9.15 (1, 91)	p <0.050

Table 31. Intact Animals: Results of univariate ANOVAs % PPI-75 dB on each enrichment day			
Males	**Effect**	**F value (d.f.)**	**p value**
ED 15	Social	1.97 1, 91)	p =0.164
	Physical	0.001 (1, 91)	p =0.973
	Social x Physical	10.58(1, 91)	P <0.050
ED 20	Social	5.07 (1, 91)	p <0.050
	Physical	0.12 (1, 91)	p =0.725
	Social x Physical	8.73(1, 91)	p <0.050
ED 30	Social	2.35 (1, 91)	p =0.129
	Physical	0.090 (1, 91)	p =0.764
	Social x Physical	1.15(1, 91)	p =0.287
Females			
ED 15	Social	0.011 (1, 91)	p =0.915
	Physical	3.33 (1, 91)	p =0.071
	Social x Physical	0.11 (1, 91)	p =0.740
ED 20	Social	0.22 (1, 91)	p =0.639
	Physical	0.33 (1, 91)	p =0.568
	Social x Physical	0.71 (1, 91)	p =0.401
ED 30	Social	5.27 (1, 91)	p <0.050
	Physical	10.69 (1, 91)	p <0.050
	Social x Physical	4.26 (1, 91)	p <0.050

Table 32. Intact Animals: Results of univariate ANOVAs % Visual on each enrichment day			
Males	**Effect**	**F value (d.f.)**	**p value**
ED 15	Social	0.38 (1, 91)	p =.537
	Physical	0.50 (1, 91)	p =.481
	Social x Physical	2.92 (1, 91)	p =.090
ED 20	Social	2.93 (1, 91)	p =.090
	Physical	11.21 (1, 91)	p <0.001
	Social x Physical	0.45 (1, 91)	p =.503
ED 30	Social	0.50 (1, 91)	p =.483
	Physical	3.02 (1, 91)	p =.086
	Social x Physical	0.03 (1, 91)	p =.861
Females			
ED 15	Social	0.11 (1, 91)	p =.745
	Physical	0.51 (1, 91)	p =.477
	Social x Physical	1.75 (1, 91)	p =.189
ED 20	Social	1.68 (1, 91)	p =.198
	Physical	1.05 (1, 91)	p =.309
	Social x Physical	1.65 (1, 91)	p =.203
ED 30	Social	0.001 (1, 91)	p =.978
	Physical	0.85 (1, 91)	p =.358
	Social x Physical	2.18 (1, 91)	p =.143

Table 33. Intact Animals: Results from Wilcoxon Signed Ranks Test on passive avoidance training latencies compared to testing latencies (females only; N =48)

Group Tested	Effect	Z value (d.f.)	p value
All Females	Time	-4.985 (48)	p <0.001
NPESE-females	Time	-1.883	p =0.060
PE-females	Time	-2.353	p <0.05
SE-females	Time	-2.668	p <0.05
PESE-females	Time	-2.903	p <0.05

Table 34. Intact Females: Results from Kruskal-Wallis nonparametric tests on passive avoidance training latencies.

Group Tested	Effect	Chi Square (d.f.)	p value
Intact females	Group	5.93 (3)	p = 0.115
	Social	2.72 (1)	p = 0.99
	Physical	0.102 (1)	p = 0.749

Table 35. Intact Females: Results from Kruskal-Wallis nonparametric tests on passive avoidance testing latencies.

Group Tested	Effect	Chi Square (d.f.)	p value
Intact females	Group	6.650 (3)	p = 0.084
	Social	5.739 (1)	p < 0.05
	Physical	0.789 (1)	p =0.375

Table 36. Intact Animals: Results of paired t-tests comparing Morris water maze averaged Trial 1 times and distances (from days 1-5; ED X-X) to averaged Trial 4 times and distances (from maze days 1-5; ED 22-26)			
Treatment Group	Comparison	t value (d.f.)	p value
Males-NPESE	Average Trial 1 time with Average Trial 4 time	7.445(23)	p <0.001
	Average Trial 1 distance with Average Trial 4 distance	5.469(23)	p <0.001
Males-PE	Average Trial 1 time with Average Trial 4 time	6.731(23)	p <0.001
	Average Trial 1 distance with Average Trial 4 distance	3.400(23)	p <0.001
Males-SE	Average Trial 1 time with Average Trial 4 time	8.999(23)	p <0.001
	Average Trial 1 distance with Average Trial 4 distance	5.523(23)	p <0.001
Males-PESE	Average Trial 1 time with Average Trial 4 time	4.497(23)	p <0.001
	Average Trial 1 distance with Average Trial 4 distance	2.872(23)	p <0.001
Females-NPESE	Average Trial 1 time with Average Trial 4 time	6.230(23)	p <0.001
	Average Trial 1 distance with Average Trial 4 distance	5.698(23)	p <0.001
Females-PE	Average Trial 1 time with Average Trial 4 time	5.954(23)	p <0.001
	Average Trial 1 distance with Average Trial 4 distance	5.680(23)	p <0.001
Females-SE	Average Trial 1 time with Average Trial 4 time	5.017(23)	p <0.001
	Average Trial 1 distance with Average Trial 4 distance	5.049(23)	p <0.001
Females-PESE	Average Trial 1 time with Average Trial 4 time	6.986(23)	p <0.001
	Average Trial 1 distance with Average Trial 4 distance	8.123(23)	p <0.001

Table 37. Intact Animals: Results of repeated-measures ANOVAs on water maze time to find platform maze days 1-5 (ED 22-26)			
Treatment Group	Effect	F value (d.f.)	p value
All Animals	Time	160.80 (4, 736)	p <0.001
	Time X Gender	6.54 (4, 736)	p <0.001
	Time X Social	3.21 (4, 736)	p <0.05
	Time X Physical	1.12 (4, 736)	p = 0.346
	Time X Gender X Social	2.48 (4, 736)	p <0.05
	Time X Gender X Physical	0.07 (4, 736)	p =.991
	Time X Social X Physical	0.55 (4, 736)	p= 0.696
	Time X Gender X Social X	1.86 (4, 736)	p = 0.116
Males	Time	140.44 (4, 368)	p <0.001
	Time X Social	5.69 (4, 368)	p <0.001
	Time X Physical	0.77(4, 368)	p = 0.546
	Time X Social X Physical	0.33 (4, 368)	p =0.855
Females	Time	45.34 (4, 368)	p <0.001
	Time X Social	0.92 (4, 368)	p = 0.451
	Time X Physical	0.48 (4, 368)	p =0.752
	Time X Social X Physical	1.79 (4, 368)	p= 0.130

Table 38. Intact Animals: Results of univariate ANOVAs on water maze time to find platform averaged across days 1-5 (enrichment days 22-26)			
Treatment Group	Effect	F value (d.f.)	p value
All Animals	Gender	20.13 (1, 184)	p <0.001
	Social	1.35 (1, 184)	p =0.246
	Physical	3.41 (1, 184)	p =0.066
	Gender X Social	0.984 (1, 184)	p =0.322
	Gender X Physical	1.414 (1, 184)	p =0.236
	Gender X Social X Physical	0.024 (1, 184)	p =0.876
Males	Social	3.509 (1, 92)	p =0.064
	Physical	0.327 (1, 92)	p = 0.569
	Social X Physical	0.119 (1, 92)	p =0.731
Females	Social	0.011 (1, 92)	p = 0.917
	Physical	3.443(1, 92)	p = 0.067
	Social X Physical	0.186 (1, 92)	p = 0.668

Table 39. Intact Animals: Results of repeated-measures ANOVAs on water maze distance traveled to find platform days 1-5 (enrichment days 22-26)			
Treatment Group	Effect	F value (d.f.)	p value
All Animals	Time	95.12 (4, 736)	p<0.001
	Time X Gender	1.33 (4, 736)	p =0.256
	Time X Social	2.17 (4, 736)	p =0.070
	Time X Physical	0.94 (4, 736)	p=.438
	Time X Gender X Social	1.33 (4, 736)	p =0.217
	Time X Gender X Physical	0.24 (4, 736)	p=.917
	Time X Social X Physical	0.85 (4, 736)	p=.494
	Time X Gender X Social X Physical	1.50 (4, 736)	p=.200
Males	Time	64.96 (4, 368)	p <0.001
	Time X Social	0.84 (4, 368)	p =0.502
	Time X Physical	1.13 (4, 368)	p =0.344
	Time X Social X Physical	0.18 (4, 368)	p =0.949
Females	Time	38.13 (4, 368)	p <0.001
	Time X Social	2.40 (4, 368)	p =0.050
	Time X Physical	0.27 (4, 368)	p =0.898
	Time X Social X Physical	1.79 (4, 368)	p =0.133

Table 40. Intact Animals: Results of univariate ANOVAs on distance traveled to find platform averaged across days 1-5 (enrichment days 22-26)			
Treatment Group	Effect	F value (d.f.)	p value
All Animals	Gender	81.68 (1, 184)	p <0.001
	Social	0.97 (1, 184)	p =0.327
	Physical	1.05 (1, 184)	p =0.307
	Social X Physical	0.002 (1, 184)	p =0.961
	Gender X Social	0.07 (1, 184)	.p =0.786
	Gender X Physical	1.82 (1, 184)	p =0.180
	Gender X Social X Physical	0.08 (1, 184)	p =0.774
Males	Social	3.51 (1, 92)	p =0.064
	Physical	0.33 (1, 92)	p = 0.569
	Social X Physical	0.12 (1, 92)	p =0.731
Females	Social	0.01 (1, 92)	p = 0.917
	Physical	3.44 (1, 92)	p = 0.067
	Social X Physical	0.19 (1, 92)	p = 0.668

Table 41. Intact Animals: Results of univariate ANOVAs on time to reach platform when all animals were considered together

Day	Effect	F value (d.f.)	p value
ED (22)	Gender	0.37 (1, 184)	p = 0.545
	Social	5.24 (1, 184)	p <0.050
	Physical	0.49 (1, 184)	p = 0.487
	Gender X Social	5.82 (1, 184)	p <0.050
	Gender X Physical	1.073 (1, 184)	p = 0.302
	Social X Physical	0.555 (1, 184)	p = 0.457
	Gender X Social X Physical	2.205 (1, 184)	p = 0.139
ED (23)	Gender	10.528(1, 184)	p <0.001
	Social	2.870(1, 184)	p = 0.092
	Physical	0.014(1, 184)	p = 0.906
	Gender X Social	1.649(1, 184)	p = 0.201
	Gender X Physical	0.262(1, 184)	p = 0.610
	Social X Physical	0.001(1, 184)	p = 0.974
	Gender X Social X Physical	0.652(1, 184)	p = 0.421
ED (24)	Gender	21.894 (1, 184)	p <0.001
	Social	0.807 (1, 184)	p = 0.370
	Physical	3.460 (1, 184)	p = 0.064
	Gender X Social	0.009 (1, 184)	p = 0.924
	Gender X Physical	0.357 (1, 184)	p = 0.551
	Social X Physical	0.735 (1, 184)	p = 0.392
	Gender X Social X Physical	1.893 (1, 184)	p = 0.171
ED (25)	Gender	12.439 (1, 184)	p <0.001
	Social	0.049 (1, 184)	p = 0.826
	Physical	6.074 (1, 184)	p < 0.050
	Gender X Social	0.945 (1, 184)	p = 0.332
	Gender X Physical	0.664 (1, 184)	p = 0.416
	Social X Physical	0.247 (1, 184)	p = 0.620
	Gender X Social X Physical	0.768 (1, 184)	p = 0.382
ED (26)	Gender	12.439 (1, 184)	p <0.001
	Social	0.049 (1, 184)	p = 0.826
	Physical	6.074 (1, 184)	p < 0.050
	Gender X Social	0.945 (1, 184)	p = 0.332
	Gender X Physical	0.664 (1, 184)	p = 0.415
	Social X Physical	0.247 (1, 184)	p = 0.620
	Gender X Social X Physical	0.768 (1, 184)	p = 0.382

Table 42. Intact Males: Results of univariate ANOVAs on time to reach platform when animals were considered separately by gender

Males	Effect	F value (d.f.)	p value
ED (22)	Social	10.605 (1, 92)	.002
	Physical	0.055 (1, 92)	.815
	Social X Physical	0.263 (1, 92)	.610
ED (23)	Social	5.795(1, 92)	.018
	Physical	0.101 (1, 92)	.752
	Social X Physical	0.392 (1, 92)	.533
ED (24)	Social	0.676 (1, 92)	.413
	Physical	1.673 (1, 92)	.199
	Social X Physical	0.282 (1, 92)	.597
ED (25)	Social	0.358 (1, 92)	.551
	Physical	1.727 (1, 92)	.192
	Social X Physical	0.091 (1, 92)	.763
ED (26)	Social	0.884 (1, 92)	.349
	Physical	0.032 (1, 92)	.859
	Social X Physical	0.102 (1, 92)	.750

Table 43. Intact Females: Results of univariate ANOVAs on time to reach platform when animals were considered separately by gender

Females	Effect	F value (d.f.)	p value
ED (22)	Social	.008 (1, 92)	.929
	Physical	1.567 (1, 92)	.214
	Social X Physical	2.595 (1, 92)	.111
ED (23)	Social	0.068 (1, 92)	.795
	Physical	0.161 (1, 92)	.689
	Social X Physical	0.286 (1, 92)	.594
ED (24)	Social	0.324 (1, 92)	.570
	Physical	1.983 (1, 92)	.162
	Social X Physical	1.637 (1, 92)	.204
ED (25)	Social	0.587 (1, 92)	.446
	Physical	4.436 (1, 92)	.038
	Social X Physical	0.778 (1, 92)	.380
ED (26)	Social	1.620 (1, 92)	.206
	Physical	2.251 (1, 92)	.137
	Social X Physical	0.003 (1, 92)	.953

Table 44. Intact Animals: Results of repeated measures ANOVAs on water maze time to find platform on Trial 1 of maze days 2-5 (enrichment days 23- 26)			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
All Animals	Time	94.22 (4, 736)	p <0.001
	Time X Gender	2.83 (4, 736)	p <0.050
	Time X Social	0.79 (4, 736)	p =0.532
	Time X Physical	1.05 (4, 736)	p =0.382
	Time X Gender X Social	1.03 (4, 736)	p =0.391
	Time X Gender X Physical	0.58 (4, 736)	p =0.681
	Time X Social X Physical	0.34 (4, 736)	p =0.851
	Time X Gender X Social X	1.02 (4, 736)	p =0.397
Males	Time	67.76 (4, 368)	p <0.001
	Time X Social	1.44 (4, 368)	p =0.221
	Time X Physical	0.95 (4, 368)	p =0.436
	Time X Social X Physical	0.87 (4, 368)	p =0.485
Females	Time	32.09 (4, 368)	p <0.001
	Time X Social	0.46 (4, 368)	p =0.766
	Time X Physical	0.69 (4, 368)	p =0.597
	Time X Social X Physical	0.52(4, 368)	p =0.721

Table 45. Intact Animals: Results of Univariate ANOVAs on time to reach platform on trail 1 averaged across days 2-5 (enrichment days 23-26)			
Treatment Group	**Effect**	**F value (d.f.)**	**p value**
All Animals	Gender	8.93 (1, 184)	p <0.050
	Social	3.57 (1, 184)	p =0.061
	Physical	3.14(1, 184)	p =0.078
	Social X Physical	1.01(1, 184)	p =0.316
	Gender X Social	0.39(1, 184)	p =0.535
	Gender X Physical	0.05(1, 184)	p =0.831
	Gender X Social X Physical	2.89(1, 184)	p =0.091
Males	Social	4.88(1, 92)	p <0.050
	Physical	3.34(1, 92)	p =0.071
	Social X Physical	2.14 (1, 92)	p =0.147
Females	Social	0.34 (1, 92)	p =0.561
	Physical	0.58 (1, 92)	p =0.449
	Social X Physical	0.97 (1, 92)	p =0.328

APPENDIX B: TABLES COMPARING INJURED VS. INTACT ANIMALS

Table 46. All Animals: Results of univariate ANOVAs on ASR amplitude comparing injured vs. intact animals at baseline			
Group Tested	**Effect**	**F value (d.f.)**	**p value**
All	Injury vs. Intact	0.006 (1, 286)	0.941
Males	Injury vs. Intact	2.300 (1, 141)	0.132
Females	Injury vs. Intact	1.736 (1, 143)	0.190

Table 47. All Animals: Results of univariate ANOVAs on ASR amplitude comparing injured vs. intact animals at baseline			
Group Tested	**Effect**	**F value (d.f.)**	**p value**
All	Injury vs. Intact	19.640 (1, 283)	p <0.001
Males	Injury vs. Intact	11.360 (1, 141)	p <0.001
Females	Injury vs. Intact	8.251 (1, 142)	p<0.05

Table 48. All Animals: Kruskal-Wallis nonparametric tests on distance to find platform for injured and non-injured animals (ED 22-26)				
Group Tested	**Effect**	**ED**	**Chi Square (d.f.)**	**p value**
	Injury vs. Intact	ED 22	7.924 (1)	.005
	Injury vs. Intact	ED 23	24.236 (1)	.000
	Injury vs. Intact	ED 24	75.156 (1)	.000
	Injury vs. Intact	ED 25	27.801 (1)	.000
	Injury vs. Intact	ED 26	27.944 (1)	.000
Males	Injury vs. Intact	ED 22	28.510 (1)	.000
	Injury vs. Intact	ED 23	0.546 (1)	.460
	Injury vs. Intact	ED 24	56.498 (1)	.000
	Injury vs. Intact	ED 25	15.093 (1)	.000
	Injury vs. Intact	ED 26	21.027 (1)	.000
Females	Injury vs. Intact	ED 22	84.645 (1)	.000
	Injury vs. Intact	ED 23	45.460 (1)	.000
	Injury vs. Intact	ED 24	31.750 (1)	.000
	Injury vs. Intact	ED 25	15.308 (1)	.000
	Injury vs. Intact	ED 26	7.473 (1)	.006

APPENDIX C: TABLES FOR EXPERIMENT II

Table 49. Injured Animals: Results of repeated-measures ANOVAs on locomotor activity (baseline to ED 28)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
All Animals	Time	5.33 (3, 246)	$p < 0.001$
	Time X Gender	2.711 (3, 246)	$p = 0.104$
	Time X Social	6.66 (3, 246)	$p < 0.001$
	Time X Physical	3.55 (3, 246)	$p < 0.050$
	Time X Gender X Social	0.039 (3, 246)	$p = 0.843$
	Time X Gender X Physical	6.44 (3, 246)	$p = .013$
	Time X Social X Physical	0.020 (3, 247)	$p = 0.887$
	Time X Gender X Social X Physical	2.54 (3, 247)	$p = 0.115$
Males	Time	0.6060(3, 247)	$p = 0.612$
	Time X Social	1.635 (3, 247)	$p = 0.184$
	Time X Physical	1.857 (3, 247)	$p = 0.140$
	Time X Social X Physical	0.402 (3, 247)	$p = 0.752$
Females	Time	6.017 (3, 247)	$p < 0.001$
	Time X Social	8.034 (3, 247)	$p < 0.001$
	Time X Physical	3.451 (3, 247)	$p < 0.050$
	Time X Social X Physical	1.049 (3, 247)	$p = 0.374$

Table 50. Injured Animals: Results of univariate ANOVAs on horizontal activity averaged across ED 12-28			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
All Animals	Gender	28.11 (1, 82)	$p < 0.001$
	Social	10.33 (1, 82)	$p < 0.050$
	Physical	3.15 (1, 82)	$p = 0.080$
	Social X Physical	0.05 (1, 82)	$p = 0.816$
	Gender X Social	0.003 (1, 82)	$p = 0.960$
	Gender X Physical	0.38 (1, 82)	$p = 0.538$
	Gender X Social X Physical	0.31(1, 82)	$p = 0.579$
Males	Social	11.43(1, 43)	$p < 0.050$
	Physical	3.20 (1, 43)	$p = 0.081$
	Social X Physical	1.33 (1, 43)	$p = 0.256$
Females	Social	2.82(1, 43)	$p = 0.101$
	Physical	0.95 (1, 43)	$p = 0.337$
	Social X Physical	0.001 (1, 43)	$p = 0.973$

Table 51. Injured Animals: Results of univariate ANOVAs on horizontal activity on each measurement day when all animals were considered together ED 12-ED 28

Day	**Effect**	**F value (d.f.)**	***p* value**
ED (12)	Gender	23.54 (1, 82)	$p < 0.001$
	Social	13.03 (1, 82)	$p < 0.001$
	Physical	5.10 (1, 82)	$p < 0.050$
	Gender X Social	2.62 (1, 82)	$p = 0.109$
	Gender X Physical	0.30 (1, 82)	$p = 0.586$
	Social X Physical	0.98 (1, 82)	$p = 0.326$
	Gender X Social X Physical	0.04 (1, 82)	$p = 0.843$
ED (17)	Gender	8.79 (1, 88)	$p < 0.050$
	Social	17.82 (1, 88)	$p < 0.001$
	Physical	7.55 (1, 88)	$p < 0.050$
	Gender X Social	0.02 (1, 88)	$p = 0.904$
	Gender X Physical	1.02 (1, 88)	$p = 0.315$
	Social X Physical	0.02 (1, 88)	$p = 0.882$
	Gender X Social X Physical	0.70 (1, 88)	$p = 0.405$
ED (28)	Gender	17.11 (1, 88)	$p < 0.001$
	Social	6.74 (1, 88)	$p < 0.050$
	Physical	1.08 (1, 88)	$p = 0.301$
	Gender X Social	0.49 (1, 88)	$p = 0.486$
	Gender X Physical	0.68(1, 88)	$p = 0.411$
	Social X Physical	1.31(1, 88)	$p = 0.255$
	Gender X Social X Physical	0.90 (1, 88)	$p = 0.345$

Table 52. Injured Males: Results of univariate ANOVAs on horizontal activity analyzed one each day ED 12-ED 28

Males	Effect	**F value (d.f.)**	***p* value**
ED (12)	Social	2.48 (1, 40)	$p = 0.255$
	Physical	4.91 (1, 40)	$p < 0.050$
	Social X Physical	0.88 (1, 40)	$p = 0.255$
ED (17)	Social	19.28 (1, 43)	$p < 0.001$
	Physical	3.09 (1, 43)	$p = 0.255$
	Social X Physical	0.99 (1, 43)	$p = 0.255$
ED (28)	Social	9.55 (1, 43)	$p < 0.050$
	Physical	0.040 (1, 43)	$p = 0.842$
	Social X Physical	0.034 (1, 43)	$p = 0.855$

Table 53. Injured Females: Results of univariate ANOVAs on horizontal activity analyzed one each day ED 12-ED 28			
Females	Effect	**F value (d.f.)**	***p* value**
ED (12)	Social	11.10 (1, 45)	p <0.050
	Physical	1.19 (1, 45)	p =0.282
	Social X Physical	0.25 (1, 45)	p =0.617
ED (17)	Social	5.72 (1, 45)	p <0.050
	Physical	4.81 (1, 45)	p <0.050
	Social X Physical	0.16 (1, 45)	p =0.690
ED (28)	Social	1.29 (1, 39)	p =0.262
	Physical	1.25 (1, 39)	p =0.270
	Social X Physical	1.58 (1, 39)	p =0.216

Table 54. Injured Animals: Results repeated-measures ANOVAs on locotomotor activity within session for injured animals (ED 12)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
All Animals	Time	210.29 (11, 902)	.000
	Time X Gender	3.85 (11, 902)	.000
	Time X Social	3.25 (11, 902)	.000
	Time X Physical	0.64 (11, 902)	.799
	Time X Gender X Social	1.45 (11, 902)	.146
	Time X Gender X Physical	1.26 (11, 902)	.246
	Time X Social X Physical	0.82 (11, 902)	.619
	Time X Gender X Social X Physical	0.61 (11, 902)	.822
Males	Time	104.133 (11, 473)	.000
	Time X Social	2.660 (11, 473)	.003
	Time X Physical	1.021 (11, 473)	.427
	Time X Social X Physical	0.761(11, 473)	.679
Females	Time	106.741 (11, 429)	.000
	Time X Social	2.084 (11, 429)	.020
	Time X Physical	0.873 (11, 429)	.567
	Time X Social X Physical	0.667 (11, 429)	.770

Table 55. Injured Animals: Results repeated-measures ANOVAs on locotomotor activity within session for injured animals (ED 17)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
All Animals	Time	247.602 (11, 968)	.000
	Time X Gender	1.591 (11, 968)	.096
	Time X Social	1.736 (11, 968)	.061
	Time X Physical	1.179 (11, 968)	.297
	Time X Gender X Social	1.920 (11, 968)	.033
	Time X Gender X Physical	0.865 (11, 968)	.575
	Time X Social X Physical	1.518 (11, 968)	.119
	Time X Gender X Social X	0.524 (11, 968)	.888
Males	Time	145.992 (11, 473)	.000
	Time X Social	2.607 (11, 473)	.003
	Time X Physical	0.764 (11, 473)	.676
	Time X Social X Physical	1.396 (11, 473)	.171
Females	Time	109.218 (11, 495)	.000
	Time X Social	1.241 (11, 495)	.257
	Time X Physical	1.233 (11, 495)	.262
	Time X Social X Physical	0.739 (11, 495)	.701

Table 56. Injured Animals: Results repeated-measures ANOVAs on locomotor activity within session for injured animals (ED 28)			
Treatment Group	Effect	**F value (d.f.)**	***p* value**
All Animals	Time	202.936 (11, 968)	.000
	Time X Gender	0.900 (11, 968)	.540
	Time X Social	1.280 (11, 968)	.231
	Time X Physical	1.473 (11, 968)	.136
	Time X Gender X Social	.609 (11, 968)	.822
	Time X Gender X Physical	1.100 (11, 968)	.357
	Time X Social X Physical	0.891(11, 968)	.549
	Time X Gender X Social X	0.936 (11, 968)	.504
Males	Time	105.109 (11, 473)	.000
	Time X Social	1.076 (11, 473)	.379
	Time X Physical	0.913 (11, 473)	.528
	Time X Social X Physical	0.385 (11, 473)	.962
Females	Time	100.116 (11, 495)	.000
	Time X Social	0.844 (11, 495)	.596
	Time X Physical	1.608 (11, 495)	.093
	Time X Social X Physical	1.359 (11, 495)	.189

Table 57. Injured Animals: Univariate ANOVAs on horizontal activity within session averaged across time period Enrichment day 12			
Treatment Group	Effect	**F value (d.f.)**	***p* value**
All Animals	Gender	23.931 (1, 82)	.000
	Social	12.702 (1, 82)	.001
	Physical	4.898 (1, 82)	.030
	Social X Physical	2.477 (1, 82)	.119
	Gender X Social	0.347 (1, 82)	.557
	Gender X Physical	0.893 (1, 82)	.348
	Gender X Social X Physical	0.058 (1, 82)	.810
Males	Social	2.475 (1, 43)	.123
	Physical	4.908 (1, 43)	.032
	Social X Physical	0.880 (1, 43)	.354
Females	Social	10.709 (1, 39)	.002
	Physical	1.070 (1, 39)	.307
	Social X Physical	0.201 (1, 39)	.657

Table 58. Injured Animals: Univariate ANOVAs on horizontal activity within session averaged across time period Enrichment day 17			
Treatment Group	Effect	**F value (d.f.)**	***p* value**
All Animals	Gender	8.920 (1, 88)	.004
	Social	17.716 (1, 88)	.000
	Physical	7.673 (1, 88)	.007
	Social X Physical	0.011 (1, 88)	.918
	Gender X Social	0.987(1, 88)	.323
	Gender X Physical	0.017(1, 88)	.896
	Gender X Social X Physical	0.672 (1, 88)	.414
Males	Social	19.188 (1, 43)	.000
	Physical	3.256 (1, 43)	.078
	Social X Physical	0.932 (1, 43)	.340
Females	Social	5.718 (1, 45)	.021
	Physical	4.805 (1, 45)	.034
	Social X Physical	0.161(1, 45)	.690

Table 59. Injured Animals: Univariate ANOVAs on horizontal activity within session averaged across time period Enrichment day 28			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
All Animals	Gender	21.284 (1, 88)	.000
	Social	6.008 (1, 88)	.016
	Physical	1.153 (1, 88)	.286
	Social X Physical	0.445 (1, 88)	.507
	Gender X Social	0.759 (1, 88)	.386
	Gender X Physical	1.345 (1, 88)	.249
	Gender X Social X Physical	0.949 (1, 88)	.333
Males	Social	9.545 (1, 43)	.004
	Physical	0.040 (1, 43)	.842
	Social X Physical	0.034 (1, 43)	.855
Females	Social	1.097 (1, 45)	.301
	Physical	1.303 (1, 45)	.260
	Social X Physical	1.569 (1, 45)	.217

Table 60. Injured Animals: Results of MANOVAs on baseline startle amplitudes and PPI values				
All Animals	**Multivariate Effect and F value (d.f.)**	**Dependent Measure**	**Univariate F value (d.f.)**	***p* value**
All animals	**Gender** 0.606 (4, 85) p =0.660	Startle to 120 db	0.52 (1, 88)	.472
		82 db pp	0.82 (1, 88)	.368
		75 db pp	0.34(1, 88)	.564
		Visual pp	0.22 (1, 88)	.639
	Group 0.894 (12, 261) p =0.553	Startle to 120 db	0.52 (3, 88)	.669
		82 db pp	0.18 (3, 88)	.908
		75 db pp	0.41 (3, 88)	.743
		Visual pp	0.89(3, 88)	.450
	Gender X Group 1.497 (12, 261) p = 0.125	Startle to 120 db	4.05 (3, 88)	.010
		82 db pp	0.39 (3, 88)	.760
		75 db pp	0.20 (3, 88)	.898
		Visual pp	1.043 (3, 88)	.378
Males	**Group** .957 (12, 126) p =0.493	Startle to 120 db	2.356 (3, 43)	.085
		82 db pp	0.04 (3, 43)	.990
		75 db pp	0.09 (3, 43)	.966
		Visual pp	1.06 (3, 43)	.378
Females	**Group** 1.516 (12, 132) p =0.134	Startle to 120 db	2.20 (3, 43)	.102
		82 db pp	0.50 (3, 43)	.687
		75 db pp	0.53 (3, 43)	.666
		Visual pp	0.90 (3,43)	.450

Table 61. Injured Animals: Results repeated-measures ANOVAs on Startle Amplitude (baseline to ED 30)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
Males	Time	57.31 (3, 129)	p <0.001
	Time X Social	1.881 (3, 129)	p =0.136
	Time X Physical	1.111 (3, 129)	p =0.347
	Time X Social X Physical	0.37 (3, 129)	p =0.775
Females	Time	9.17 (3, 129)	p <0.001
	Time X Social	0.65 (3, 129)	p =0.585
	Time X Physical	0.26 (3, 129)	p =0.853
	Time X Social X Physical	0.52 (3, 129)	p =0.667

Table 62. Injured Animals: Results Univariate ANOVAs startle amplitude averaged across days (baseline to ED 30)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
Males	Social	.98 (1, 43)	p =0.327
	Physical	.77 (1, 43)	p =0.385
	Social x Physical	.70 (1, 43)	p =0.408
Females	Social	.50 (1, 43)	p =0.485
	Physical	6.38 (1, 43)	p<0.050
	Social x Physical	3.09 (1, 43)	p =0.086

Table 63. Injured Animals: Results repeated-measures ANOVAs on % PPI-82 (baseline-ED 30)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
Males	Time	3.37 (3, 129)	p<0.050
	Time X Social	0.73 (3, 129)	p =0.536
	Time X Physical	3.16 (3, 129)	p<0.050
	Time X Social X Physical	0.11 (3, 129)	p =0.952
Females	Time	1.45 (3,132)	p =0.231
	Time X Social	0.76 (3,132)	p =0.516
	Time X Physical	0.36 (3,132)	p =0.782
	Time X Social X Physical	0.24 (3,132)	p =0.868

Table 64. Injured Animals: Results Univariate ANOVAs % PPI-82 dB averaged across baseline-ED 30			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
Males	Social	0.12 (1, 43)	*p* =0.727
	Physical	0.26 (1, 43)	*p* =0.616
	Social x Physical	0.09 (1, 43)	*p* =0.763
Females	Social	2.21 (1, 44)	*p* =0.145
	Physical	2.73 (1, 44)	*p* =0.105
	Social x Physical	0.14 (1, 44)	*p* =0.713

Table 65. Injured Animals: Results repeated-measures ANOVAs on % PPI-75 (baseline-ED 30)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
Males	Time	1.28 (3, 129)	*p* =0.285
	Time X Social	0.47(3, 129)	*p* =0.707
	Time X Physical	1.69 (3, 129)	*p* =0.173
	Time X Social X	0.25 (3, 129)	*p* =0.860
Females	Time	0.34 (3, 132)	*p* =0.799
	Time X Social	0.84 (3, 132)	*p* =0.475
	Time X Physical	0.36 (3, 132)	*p* =0.785
	Time X Social X	2.88 (3, 132)	*p* <0.050

Table 66. Injured Animals: Results Univariate ANOVAs % PPI-75 dB averaged across baseline-ED 30			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
Males	Social	0.21 (1, 43)	*p* =0.647
	Physical	0.11 (1, 43)	*p* =0.743
	Social x Physical	0.25(1, 43)	*p* =0.622
Females	Social	1.99 (1, 44)	*p* =0.166
	Physical	1.12 (1, 44)	*p* =0.295
	Social x Physical	0.72(1, 44)	*p* =0.401

Table 67. Injured Animals: Results repeated-measures ANOVAs on % visual PPI (baseline–ED 30)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
Males	Time	1.322 (3, 129)	*p* = .270
	Time X Social	0.574 (3, 129)	*p* =0.633
	Time X Physical	1.922 (3, 129)	*p* = 0.129
	Time X Social X Physical	.393(3, 129)	*p* =0.758
Females	Time	0.555 (3, 132)	*p* =0 .646
	Time X Social	0.547(3, 132)	*p* =0.651
	Time X Physical	0.994 (3, 132)	*p* =0.398
	Time X Social X Physical	1.534 (3, 132)	*p* =0.209

Table 68. Injured Animals: Results Univariate ANOVAs % visual PPI averaged across baseline to ED 30			
Treatment Group	Effect	**F value (d.f.)**	***p* value**
Males	Social	0.22 (1, 43)	*p* =0.882
	Physical	0.002 (1, 43)	*p* =0.966
	Social x Physical	3.848 (1, 43)	*p* =0.056
Females	Social	2.074 (1, 44)	*p* =0.157
	Physical	0.123 (1, 44)	*p* =0.727
	Social x Physical	0.99 (1, 44)	*p* =0.754

Table 69. Injured Animals: Results of Kruskal-Wallis nonparametric tests on PA training latencies.			
Group Tested	**Effect**	**Chi Square (d.f.)**	***p* value**
All animals	Group	9.956 (3)	*p* =<0.050
	Social	5.007 (1)	*p* =<0.050
	Physical	4.894 (1)	*p* =<0.050
Males	Group	5.340 (3)	*p* =0.149
	Social	4.010 (1)	*p* =<0.050
	Physical	.800 (1)	*p* =0.371
Females	Group	6.952 (3)	*p* =0.073
	Social	1.265 (1)	*p* =0.261
	Physical	5.735 (1)	*p* <0.050

Table 70. Injured Animals: Results of Kruskal-Wallis nonparametric tests on PA testing latencies.			
Group Tested	**Effect**	**Chi Square (d.f.)**	***p* value**
All animals	Group	1.785 (3)	*p* =0.682
	Social	.906(1)	*p* =0.341
	Physical	.862 (1)	*p* =0.353
Males	Group	1.040 (3)	*p* =0.792
	Social	0.197 (1)	*p* =0.657
	Physical	.152 (1)	*p* =0.696
Females	Group	5.109 (3)	*p* =0.164
	Social	3.689 (1)	*p* =<0.050
	Physical	.884 (1)	*p* =0.347

Table 71. Injured Animals: Results from Wilcoxon Signed Ranks Test on passive avoidance training latencies compared to testing latencies			
Group Tested	**Effect**	**Z value (d.f.)**	**p value**
All Animals	Time	-7.614 (88)	p <0.001
Males	Time	-5.345 (42)	p <*0.001*
Females	Time	-5.414(46)	p <*0.001*
NPESE-males	Time	2.845 (11)	*p* <0.050
PE-males	Time	2.934 (11)	*p* <0.050
SE-males	Time	2.045 (11)	*p* <0.050
PESE-males	Time	2.668 (09)	*p* <0.050
NPESE-females	Time	2.621 (13)	*p* <0.050
PE-females	Time	2.353 (12)	*p* <0.050
SE-females	Time	2.803 (10)	*p* <0.050
PESE-females	Time	2.845 (11)	*p* <0.050

Table 72. Injured Animals: Results of paired t-tests comparing Morris water maze averaged Trial 1 times and distances (from days 1-5; ED X-X) to averaged Trial 4 times and distances (from days 1-5; ED X-X)

Treatment Group	**Comparison**	**t value (d.f.)**	***p* value**
Males-NPESE	Average Trial 1 time with Average Trial 4 time	8.019 (11)	*p* <0.001
	Average Trial 1 distance with Average Trial 4	3.099(11)	*p* <0.001
Males-PE	Average Trial 1 time with Average Trial 4 time	8.349 (10)	*p* <0.001
	Average Trial 1 distance with Average Trial 4	3.020 (10)	*p* <0.001
Males-SE	Average Trial 1 time with Average Trial 4 time	7.212 (11)	*p* <0.001
	Average Trial 1 distance with Average Trial 4	4.259 (11)	*p* <0.001
Males-PESE	Average Trial 1 time with Average Trial 4 time	4.756 (11)	*p* <0.001
	Average Trial 1 distance with Average Trial 4	3.525 (11)	*p* <0.001
Females-NPESE	Average Trial 1 time with Average Trial 4 time	3.579 (13)	*p* <0.001
	Average Trial 1 distance with Average Trial 4	3.863 (13)	*p* <0.001
Females-PE	Average Trial 1 time with Average Trial 4 time	5.688 (11)	*p* <0.001
	Average Trial 1 distance with Average Trial 4	4.682 (11)	*p* <0.001
Females-SE	Average Trial 1 time with Average Trial 4 time	4.003 (10)	*p* <0.001
	Average Trial 1 distance with Average Trial 4	3.090 (10)	*p* <0.001
Females-PESE	Average Trial 1 time with Average Trial 4 time	6.857 (11)	*p* <0.001
	Average Trial 1 distance with Average Trial 4	6.476 (11)	*p* <0.001

Table 73. Injured Animals: Results of repeated-measures ANOVAs on water maze time to find platform days 1-5 (enrichment days 22-26)

Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
All Animals	Time	65.97 (4, 352)	*p* <0.001
	Time X Gender	15.987 (4, 352)	*p* <0.001
	Time X Social	1.017 (4, 352)	*p* =0.398
	Time X Physical	.470 (4, 352)	*p* =0.758
	Time X Gender X Social	1.255 (4, 352)	*p* =0.287
	Time X Gender X Physical	1.535 (4, 352)	*p* =0.191
	Time X Social X Physical	.182 (4, 352)	*p* =0.948
	Time X Gender X Social X	.287 (4, 352)	*p* =0.886
Males	Time	14.814 (4, 172)	*p* <0.001
	Time X Social	2.093 (4, 172)	*p* =0.084
	Time X Physical	.696 (4, 172)	*p* =0.595
	Time X Social X Physical	.311 (4, 172)	*p* =0.870
Females	Time	65.579 (4, 180)	*p* <0.001
	Time X Social	.248 (4, 180)	*p* =0.911
	Time X Physical	1.292 (4, 180)	*p* =0.275
	Time X Social X Physical	.163 (4, 180)	*p* =0.957

Table 74. Injured Animals: Results of univariate ANOVAs on water maze time to find platform averaged across days 1-5 (enrichment days 22-26)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
All Animals	Gender	10.06 (1, 88)	*p* <0.050
	Social	3.01 (1, 88)	*p* =0.086
	Physical	2.97 (1, 88)	*p* =0.088
	Gender X Social	0.40 (1, 88)	*p* =0.529
	Gender X Physical	0.98 (1, 88)	*p* =0.325
	Gender X Social X Physical	3.15 (1, 88)	*p* =0.080
Males	Social	0.52 (1. 43)	*p* =0.476
	Physical	3.13 (1. 43)	*p* =0.084
	Social X Physical	0.92(1. 43)	*p* =0.344
Females	Social	3.35 (1, 45)	*p* =0.074
	Physical	0.32 (1, 45)	*p* =0.573
	Social X Physical	2.58 (1, 45)	*p* =0.115

Table 75. Injured Animals: Results of repeated-measures ANOVAs on water maze distance traveled to find platform days 1-5 (enrichment days 22-26)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
All Animals	Time	21.70 (4, 328)	*p* <0.001
	Time X Gender	41.34(4, 328)	*p* <0.001
	Time X Social	0.18 (4, 328)	*p* =0.950
	Time X Physical	0.36 (4, 328)	*p* =0.837
	Time X Gender X Social	0.66 (4, 328)	*p* =0.620
	Time X Gender X Physical	1.06 (4, 328)	*p* =0.377
	Time X Social X Physical	0.17 (4, 328)	*p* =0.956
	Time X Gender X Social X	0.54 (4, 328)	*p* =0.703
Males	Time	3.99 (4, 148)	*p* <0.050
	Time X Social	0.97 (4, 148)	*p* =0.727
	Time X Physical	0.86 (4, 148)	*p* =0.490
	Time X Social X Physical	0.13 (4, 148)	*p* =0.973
Females	Time	53.56 (4, 180)	*p* <0.001
	Time X Social	0.08 (4, 180)	*p* =0.360
	Time X Physical	0.68(4. 180)	*p* =0.610
	Time X Social X Physical	0.547 (4, 180)	*p* =0.701

Table 76. Injured Animals: Results for univariate ANOVAs on distance traveled to find platform averaged across days 1-5 (enrichment days 22-26)			
Treatment Group	**Effect**	**F value (d.f.)**	***p* value**
All Animals	Gender	81.99 (1, 82)	$p < 0.001$
	Social	0.02 (1, 82)	$p = 0.899$
	Physical	0.02 (1, 82)	$p = 0.897$
	Social X Physical	2.67 (1, 82)	$p = 0.106$
	Gender X Social	0.17 (1, 82)	$p = 0.683$
	Gender X Physical	2.35 (1, 82)	$p = 0.129$
	Gender X Social X Physical	1.25 (1, 82)	$p = 0.267$
Males	Social	1.42 (1, 37)	$p = 0.241$
	Physical	0.04 (1, 37)	$p = 0.850$
	Social X Physical	0.08 (1, 37)	$p = 0.780$
Females	Social	1.25 (1, 45)	$p = 0.269$
	Physical	0.16 (1, 45)	$p = 0.690$
	Social X Physical	3.88 (1, 45)	$p < 0.050$